

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
21 November 2002 (21.11.2002)

PCT

(10) International Publication Number
WO 02/092015 A2(51) International Patent Classification⁷: **A61K**

(21) International Application Number: PCT/US02/15982

(22) International Filing Date: 17 May 2002 (17.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/291,311	17 May 2001 (17.05.2001)	US
60/353,058	1 February 2002 (01.02.2002)	US
60/361,293	4 March 2002 (04.03.2002)	US

(71) Applicants (for all designated States except US):
GENOME THERAPEUTICS CORPORATION [US/US]; 100 Beaver Street, Waltham, MA 02453 (US).
WYETH [US/US]; Five Giralda Farms, Madison, NJ 07928 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ALLEN, Kristina** [US/US]; 11 Oliver Lane, Hopkinton, MA 01748-3108

(US). **ANISOWICZ, Anthony** [US/US]; 50 Upham Street, West Newton, MA 02465 (US). **BHAT, Bheem, M.** [IN/US]; 1214 Mayapple Lane, West Chester, PA 19380 (US). **DAMAGNEZ, Veronique** [FR/US]; 125 Water Street, Framingham, MA 01701 (US). **ROBINSON, John, Allen** [US/US]; 23 Webb Road, Downingtown, PA 19335 (US). **YAWORSKY, Paul, J.** [US/US]; 13 Hobart Lane, Rockland, MA 02370 (US).

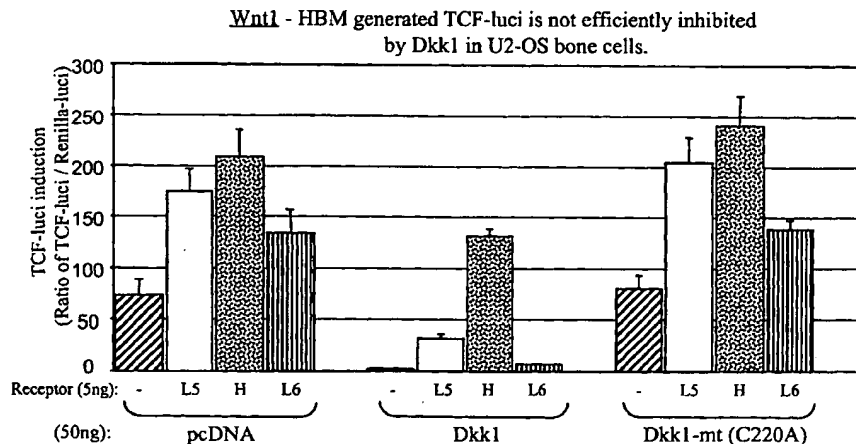
(74) Agents: **REA, Teresa, Stanek et al.**; Burns, Doane, Swecker & Mathis L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),

[Continued on next page]

(54) Title: REAGENTS AND METHODS FOR MODULATING DKK-MEDIATED INTERACTIONS



- With Wnt1 the TCF-signal generated by LRP5 is greater than that of LRP6.
- LRP5/6 -Wnt1 induced TCF- is efficiently blocked by Dkk1

(57) Abstract: The present invention provides reagents, compounds, compositions, and methods relating to novel interactions of the extracellular domain of LRP5, HBM (a variant of LRP5), and/or LRP6 with Dkk, including Dkk-1. The various nucleic acids, polypeptides, antibodies, assay methods, diagnostic methods, and methods of treatment of the present invention are related to and impact on Dkk, LRP5, LRP6, HBM, and Wnt signaling. Dkk, LRP5, LRP6, HBM, and Wnt are implicated in bone and lipid cellular signaling. Thus, the present invention provides reagents and methods for modulating lipid levels and/or bone mass and is useful in the treatment and diagnosis of abnormal lipid levels and bone mass disorders, such as osteoporosis.



Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

- *without international search report and to be republished
upon receipt of that report*

REAGENTS AND METHODS FOR MODULATING DKK-MEDIATED INTERACTIONS

FIELD OF THE INVENTION

The present invention relates to signal transduction, bone development, bone
5 loss disorders, modulation of lipid-related conditions, research reagents, methods of
screening drug leads, drug development, treatments for bone and/or lipid disorders,
screening and development of therapies, molecular, cellular, and animal models of
bone and/or lipid development and maintenance, which are mediated by Dkk,
including Dkk-1, and/or LRP5, LRP6, HBM or other members of the Wnt pathway.

BACKGROUND OF THE INVENTION

Two of the most common types of osteoporosis are postmenopausal and
senile osteoporosis. Osteoporosis affects both men and women, and, taken with
other abnormalities of bone, presents an ever-increasing health risk for an aging
15 population. The most common type of osteoporosis is that associated with
menopause. Most women lose between 20-60% of the bone mass in the trabecular
compartment of the bone within 3-6 years after the cessation of menses. This rapid
bone loss is generally associated with an increase of bone resorption and formation.
However, the resorptive cycle is more dominant and the result is a net loss of bone
20 mass. Osteoporosis is a common and serious disease among postmenopausal
women. There are an estimated 25 million women in the United States alone who
are afflicted with this disease. The results of osteoporosis are personally harmful,
and also account for a large economic loss due to its chronicity and the need for
extensive and long-term support (e.g., hospitalization and nursing home care) from
25 disease sequelae. This is especially true in elderly patients. Additionally, while
osteoporosis is generally not thought of as a life-threatening condition, a 20-30%
mortality rate is related to hip fractures in elderly women. A large percentage of this
mortality rate can be directly associated with postmenopausal osteoporosis.

The most vulnerable tissue in the bone to the effects of postmenopausal osteoporosis is the trabecular bone. This tissue is often referred to as spongy bone and is particularly concentrated near the ends of the bone, near the joints, and in the vertebrae of the spine. The trabecular tissue is characterized by small structures which inter-connect with each other as well as the more solid and dense cortical tissue which makes up the outer surface and central shaft of the bone. This criss-cross network of trabeculae gives lateral support to the outer cortical structure and is critical to the biomechanical strength of the overall structure. In postmenopausal osteoporosis, it is primarily the net resorption and loss of the trabeculae which lead to the failure and fracture of the bone. In light of the loss of the trabeculae in postmenopausal women, it is not surprising that the most common fractures are those associated with bones which are highly dependent on trabecular support, e.g., the vertebrae, the neck of the femur, and the forearm. Indeed, hip fracture, Colle's fractures, and vertebral crush fractures are indicative of postmenopausal osteoporosis. Osteoporosis affects cortical as well as trabecular bone. Alterations in endosteal bone resorption and Haversian remodeling with age affect cortical thickness and structural integrity contributing the increased risk for fracture.

One of the earliest generally accepted methods for treatment of postmenopausal osteoporosis was estrogen replacement therapy. Although this therapy frequently is successful, patient compliance is low, primarily due to the undesirable side-effects of chronic estrogen treatment. Frequently cited side-effects of estrogen replacement therapy include reinitiation of menses, bloating, depression, and, potentially, increased risk of breast or uterine cancer. In order to limit the known threat of uterine cancer in women who have not had a hysterectomy, a protocol of estrogen and progestin cyclic therapy is often employed. This protocol is similar to that used in birth control regimens, and often is not tolerated by women because of the side-effects characteristic of progestin. More recently, certain antiestrogens, originally developed for the treatment of breast cancer, have been shown in experimental models of postmenopausal osteoporosis to be efficacious. Among these agents is raloxifene (See, U.S. Patent No. 5,393,763; Black *et al.*, J.

Clin. Invest., 93:63-69 (1994); and Ettinger *et al.*, *JAMA* 282:637-45 (1999)). In addition, tamoxifen, a widely used clinical agent for treating breast cancer, has been shown to increase bone mineral density in post menopausal women suffering from breast cancer (Love *et al.*, *N. Engl. J. Med.*, 326:852-856 (1992)).

5 Another therapy for the treatment of postmenopausal osteoporosis is the use of calcitonin. Calcitonin is a naturally occurring peptide which inhibits bone resorption and has been approved for this use in many countries (Overgaard *et al.*, *Br. Med. J.*, 305:556-561 (1992)). The use of calcitonin has been somewhat limited, however. Its effects are very modest in increasing bone mineral density, and the
10 treatment is very expensive. Another therapy for the treatment of postmenopausal osteoporosis is the use of bisphosphonates. These compounds were originally developed for treating Paget's disease and malignant hypercalcemia. They have been shown to inhibit bone resorption. Alendronate, a bisphosphonate, has been approved for the treatment of postmenopausal osteoporosis. These agents may be
15 helpful in the treatment of osteoporosis, but these agents also have potential liabilities which include osteomalacia, extremely long half-life in bone (greater than 2 years), and possible "frozen bone syndrome," e.g., the cessation of normal bone remodeling.

20 Senile osteoporosis is similar to postmenopausal osteoporosis in that it is marked by the loss of bone mineral density and resulting increase in fracture rate, morbidity, and associated mortality. Generally, it occurs in later life, *i.e.*, after 70 years of age. Historically, senile osteoporosis has been more common in females, but with the advent of a more elderly male population, this disease is becoming a major factor in the health of both sexes. It is not clear what, if any, role hormones
25 such as testosterone or estrogen have in this disease, and its etiology remains obscure. Treatment of this disease has not been very satisfactory. Hormone therapy, estrogen in women and testosterone in men, has shown equivocal results; calcitonin and bisphosphonates may be of some utility.

30 The peak mass of the skeleton at maturity is largely under genetic control. Twin studies have shown that the variance in bone mass between adult monozygotic

twins is smaller than between dizygotic twins (Slemenda *et al.*, *J. Bone Miner. Res.*, 6: 561-567 (1991); Young *et al.*, *J. Bone Miner. Res.*, 6:561-567 (1995); Pocock *et al.*, *J. Clin. Invest.*, 80:706-710 (1987); Kelly *et al.*, *J. Bone Miner. Res.*, 8:11-17 (1993)). It has been estimated that up to 60% or more of the variance in skeletal mass is inherited (Krall *et al.*, *J. Bone Miner. Res.*, 10:S367 (1993)). Peak skeletal mass is the most powerful determinant of bone mass in elderly years (Hui *et al.*, *Ann. Int. Med.*, 111:355-361 (1989)), even though the rate of age-related bone loss in adult and later life is also a strong determinant (Hui *et al.*, *Osteoporosis Int.*, 1:30-34 (1995)). Since bone mass is the principal measurable determinant of fracture risk, the inherited peak skeletal mass achieved at maturity is an important determinant of an individual's risk of fracture later in life. Thus, study of the genetic basis of bone mass is of considerable interest in the etiology of fractures due to osteoporosis.

Recently, a strong interest in the genetic control of peak bone mass has developed in the field of osteoporosis. The interest has focused mainly on candidate genes with suitable polymorphisms to test for association with variation in bone mass within the normal range, or has focused on examination of genes and gene loci associated with low bone mass in the range found in patients with osteoporosis. The vitamin D receptor locus (VDR) (Morrison *et al.*, *Nature*, 367:284-287 (1994)), PTH gene (Howard *et al.*, *J. Clin. Endocrinol. Metab.*, 80:2800-2805 (1995); Johnson *et al.*, *J. Bone Miner. Res.*, 8:11-17 (1995); Gong *et al.*, *J. Bone Miner. Res.*, 10:S462 (1995)) and the estrogen receptor gene (Hosoi *et al.*, *J. Bone Miner. Res.*, 10:S170 (1995); Morrison *et al.*, *Nature*, 367:284-287 (1994)) have figured most prominently in this work. These studies are difficult because bone mass (*i.e.*, the phenotype) is a continuous, quantitative, polygenic trait, and is confounded by environmental factors such as nutrition, co-morbid disease, age, physical activity, and other factors. Also, this type of study design requires large numbers of subjects. In particular, the results of VDR studies to date have been confusing and contradictory (Garnero *et al.*, *J. Bone Miner. Res.*, 10:1283-1288 (1995); Eisman *et al.*, *J. Bone. Miner. Res.*, 10:1289-1293 (1995); Peacock, *J. Bone Miner. Res.*, 10:1294-1297 (1995)).

Furthermore, thus far, the art has not determined the mechanism(s) whereby the genetic influences exert their effect on bone mass.

While it is well known that peak bone mass is largely determined by genetic rather than environmental factors, studies to determine the gene loci (and ultimately the genes) linked to variation in bone mass are difficult and expensive. Study designs which utilize the power of linkage analysis, e.g., sib-pair or extended family, are generally more informative than simple association studies, although the latter do have value. However, genetic linkage studies involving bone mass are hampered by two major problems. The first problem is the phenotype, as discussed briefly above. Bone mass is a continuous, quantitative trait, and establishing a discrete phenotype is difficult. Each anatomical site for measurement may be influenced by several genes, many of which may be different from site to site. The second problem is the age component of the phenotype. By the time an individual can be identified as having low bone mass, there is a high probability that their parents or other members of prior generations will be deceased and therefore unavailable for study, and younger generations may not have even reached peak bone mass, making their phenotyping uncertain for genetic analysis.

Thus, there is a need in the art for additional research tools for the elucidation of the molecular mechanism of bone modulation, for the screening and development of candidate drugs, and for treatments of bone development and bone loss disorders. The present invention is directed to these, as well as other, important ends.

In addition to bone modulation, the present invention relates to modulation of lipid levels. Cardiovascular disease is the most common cause of mortality in the United States, and atherosclerosis is the major cause of heart disease and stroke. It is widely appreciated that cholesterol plays an important role in atherogenesis. Normally, most cholesterol serves as a structural element in the walls of cells, whereas much of the rest is in transit through the blood or functions as the starting material for the synthesis of bile acids in the liver, steroid hormones in endocrine cells and vitamin D in skin. The transport of cholesterol and other lipids through the

circulatory system is facilitated by their packaging into lipoprotein carriers. These spherical particles comprise protein and phospholipid shells surrounding a core of neutral lipid, including unesterified ("free") or esterified cholesterol and triglycerides. Risk for atherosclerosis increases with increasing concentrations of low density lipoprotein (LDL) cholesterol, whereas risk is inversely proportional to levels of high-density lipoprotein (HDL) cholesterol. The receptor-mediated control of plasma LDL levels has been well-defined, and recent studies have now provided new insights into HDL metabolism.

The elucidation of LDL metabolism began in 1974 by Michael Brown and Joseph Goldstein. In brief, the liver synthesizes a precursor lipoprotein (very low density lipoprotein, VLDL) that is converted during circulation to intermediate density lipoprotein (IDL) and then to LDL. The majority of the LDL receptors expressed in the body are on the surfaces of liver cells, although virtually all other tissues ("peripheral tissues") express some LDL receptors. After binding, the receptor-lipoprotein complex is internalized by the cells via coated pits and vesicles, and the entire LDL particle is delivered to lysosomes, wherein it is disassembled by enzymatic hydrolysis, releasing cholesterol for subsequent cellular metabolism. This whole-particle uptake pathway is called "receptor-mediated endocytosis." Cholesterol-mediated feedback regulation of both the levels of LDL receptors and cellular cholesterol biosynthesis help ensure cellular cholesterol homeostasis. Genetic defects in the LDL receptor in humans results in familial hypercholesterolemia, a disease characterized by elevated plasma LDL cholesterol and premature atherosclerosis and heart attacks. One hypothesis for the deleterious effects of excess plasma LDL cholesterol is that LDL enters the artery wall, is chemically modified, and then is recognized by a special class of receptors called macrophage scavenger receptors, that mediate the cellular accumulation of the LDL cholesterol in the artery, eventually leading to the formation of an atherosclerotic lesion.

The major lipoprotein classes include intestinally derived chylomicrons that transport dietary fats and cholesterol, hepatic-derived VLDL, IDL, and LDL that can be atherogenic, and hepatic- and intestinally-derived HDL that are antiatherogenic.

Apoprotein B (ApoB) is necessary for the secretion of chylomicrons (ApoB48) and VLDL, IDL, and LDL (ApoB100). Plasma levels of VLDL triglycerides are determined mainly by the rates of secretion in LDL lipolytic activity. Plasma levels of LDL cholesterol are determined mainly by the secretion of ApoB100 into plasma, the efficacy with which VLDL are converted to LDL and by LDL receptor-mediated clearance. Regulation of HDL cholesterol levels is complex and is affected by rates of synthesis of its Apo proteins, rates of esterification of free cholesterol to cholesterol ester by LCAT, levels of triglyceride-rich lipoproteins and CETP-mediated transfer of cholesterol esters from HDL, and clearance from plasma of HDL lipids and Apo proteins.

Normal lipoprotein transport is associated with low levels of triglycerides and LDL cholesterol and high levels of HDL cholesterol. When lipoprotein transport is abnormal, lipoprotein levels can change in ways that predispose individuals to atherosclerosis and arteriosclerosis (see Ginsburg, *Endocrinol. Metab. Clin. North Am.*, 27:503-19 (1998)).

Several lipoprotein receptors may be involved in cellular lipid uptake. These receptors include: scavenger receptors; LDL receptor-related protein/ α 2-macroglobulin receptor (LRP); LDL receptor; and VLDL receptor. With the exception of the LDL receptor, all of these receptors are expressed in atherosclerotic lesions while scavenger receptors are mostly expressed in macrophages, the LRP and VLDL receptors may play an important role in mediating lipid uptake in smooth muscle cells (Hiltunen *et al.*, *Atherosclerosis*, 137 suppl.:S81-8 (1998)).

A major breakthrough in the pharmacologic treatment of hypercholesterolemia has been the development of the "statin" class of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG CoA reductase) inhibitory drugs. 3-hydroxy-3-methylglutaryl-CoA reductase is the rate controlling enzyme in cholesterol biosynthesis, and its inhibition in the liver stimulates LDL receptor expression. As a consequence, both plasma LDL cholesterol levels and the risk for atherosclerosis decrease. The discovery and analysis of the LDL receptor system has had a profound impact on cell biology, physiology, and medicine.

HDL is thought to remove unesterified, or "free" cholesterol (FC) from peripheral tissues, after which most of the cholesterol is converted to cholesterol ester (CE) by enzymes in the plasma. Subsequently, HDL cholesterol is efficiently delivered directly to the liver and steroidogenic tissues via a selective uptake
5 pathway and the HDL receptor, SR-BI (class B type I scavenger receptor) or, in some species, transferred to other lipoproteins for additional transport in metabolism (see Krieger, *Proc. Natl. Acad. Sci. USA*, 95:4077-4080 (1998)).

These issues illustrate a need in the art for additional research tools for the elucidation of the molecular mechanism of lipid modulation, for the screening and
10 development of candidate drugs, and for treatments of lipid levels and lipid level modulation disorders. The present invention is directed to these, as well as other, important ends.

SUMMARY OF THE INVENTION

15 The present invention provides reagents, compounds, compositions and methods relating to novel interactions of the extracellular domain of LRP5, HBM (a variant of LRP5), and/or LRP6 with Dkk proteins. LRP5 is also referred to as Zmax1 or Zmax. Thus, when discussing methods, reagents, compounds, and compositions of the invention which relate to the interaction between Dkk and LRP5 (or Zmax1),
20 the invention is also to be understood to encompass embodiments relating to interactions between Dkk and LRP6 and Dkk and HBM. Moreover, where Dkk is discussed herein, it is to be understood that the methods, reagents, compounds, and compositions of the present invention include the Dkk family members, including but not limited to Dkk-1, Dkk-2, Dkk-3, Dkk-4 and Soggy. Furthermore, the invention
25 encompasses novel fragments of Dkk-1 which demonstrate a binding interaction between the ligand binding domain (LBD) of LRP5 and additional proteins and/or which can modulate an interaction between LRP5, or a variant or fragment thereof, and a Dkk protein. The invention provides assays, methods, compositions, and compounds relating to Dkk-Wnt signaling. Numerous Wnt proteins are compatible
30 with the present invention, including Wnt1-Wnt19, and particularly, Wnt1, Wnt3,

Wnt3a, and Wnt10b. The present invention further provides reagents, compounds, compositions and methods modulating interactions between one or more other proteins and Dkk-1. The present invention also provides a series of peptide aptamers which bind to Dkk-1 or to LRP5 (or HBM and/or LRP6).

5 The polypeptides of the invention, for example in the form of peptide oligomers, aptamers, proteins, and protein fragments as well as the nucleic acids of the invention, for example in the form of nucleic acids which encode the polypeptides of the invention as well as antisense, or complimentary nucleic acids, are useful as reagents for the study of bone mass and lipid level modulation. The polypeptides
10 and nucleic acids of the invention are also useful as therapeutic and diagnostic agents.

 The present invention provides useful reagents for the modulation of Dkk proteins with LRP5, LRP6, and/or HBM, the modulation Dkk-1 and/or Dkk-1
15 interacting protein activity, and modulation of LRP5/Dkk-1, LRP6/Dkk1 and HBM/Dkk-1 interactions and Dkk-1/Dkk-1 interacting protein interactions. The present invention provides a series of peptide aptamers which bind Dkk-1 or LRP5, LRP6, and/or HBM.

 An object of the invention is to provide for a method of regulating LRP5/LRP6/HBM/HBM-like activity in a subject comprising administering a
20 therapeutically effective amount of a composition which modulates Dkk activity. The subject can be a vertebrate or an invertebrate organism, but more preferably the organism is a canine, a feline, an ovine, a primate, an equine, a porcine, a caprine, a camelid, an avian, a bovine, or a rodent organism. A more preferred organism is a human. In a preferred embodiment, the Dkk protein is Dkk-1. In a particularly
25 preferred embodiment, Dkk-1 activity is decreased. In another embodiment, Dkk activity modulates bone mass and/or lipid levels. In a preferred embodiment, bone mass is increased and/or lipid levels are decreased. In another preferred embodiment, the modulation in bone mass is an increase in bone strength determined via one or more of a decrease in fracture rate, an increase in areal bone
30 density, an increase in volumetric mineral bone density, an increase in trabecular

connectivity, an increase in trabecular density, an increase in cortical density or thickness, an increase in bone diameter, and an increase in inorganic bone content. The invention further provides such a method wherein the composition comprises a Dkk, Dkk-1 or a LRP5/LRP6/HBM binding fragment thereof, such as those depicted in Figure 6 or a mimetic of those fragments depicted in Figure 6. The invention further provides such a method wherein the composition comprises one or more of the proteins which interact with Dkk, including Dkk-1, such as those depicted in Figure 5, or a Dkk-binding fragment thereof, or an antisense, siRNA, or shRNA molecule which recognizes and binds to a nucleic acid encoding one or more Dkk interacting or Dkk-1 interacting proteins. The invention further provides such a method wherein the composition comprises an LRP5/LRP6/Zmax1 antibody, Dkk antibody, a Dkk-1 antibody or an antibody to a Dkk-1 interacting protein. The invention further provides such a method wherein the compositions comprise an aptamer of Dkk or Dkk-1, such as those depicted in Figure 3 (SEQ ID NOs:171-188), or a mimetic of such an aptamer. The method further provides that invention further provides such a method wherein the compositions comprise an aptamer of a Dkk interacting or Dkk-1 interacting protein, or a mimetic of such an aptamer.

A composition of the present invention may modulate activity either by enhancing or inhibiting the binding of Dkk to LRP5/LRP6/Zmax1, particularly Dkk-1, or the binding of Dkk-1 to a Dkk-1 interacting protein, such as those shown in Figure 5. A composition of the present invention may comprise an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208), Figures 4 (SEQ ID NOs:189-192) (particularly, peptide (SEQ ID NO:191) and 13 (including SEQ IDNOs:204-214), or a mimetic of such an aptamer. Preferred compositions of the present invention also comprise LRP5 antibodies.

Another aspect of the invention is to provide for a method of regulating Dkk-Wnt pathway activity in a subject comprising administering a therapeutically effective amount of a composition which modulates Dkk-Wnt pathway activity. In a preferred embodiment, the Dkk protein is Dkk-1. In a particularly preferred embodiment, Dkk-1 activity is decreased. In another embodiment, Dkk activity modulates bone mass

and/or lipid levels. In a preferred embodiment, bone mass is increased and/or lipid levels are decreased. In another preferred embodiment, the modulation in bone mass is an increase in bone strength determined via one or more of a decrease in fracture rate, an increase in areal bone density, an increase in volumetric mineral bone density, an increase in trabecular connectivity, an increase in trabecular density, an increase in cortical density or thickness, an increase in bone diameter, and an increase in inorganic bone content. In another preferred embodiment, the Wnt is Wnt1-Wnt19. In a particularly preferred embodiment, the Wnt is Wnt1, Wnt3, Wnt3a, or Wnt10b. Preferred compositions comprise Dkk-modulating or Dkk-1-modulating compounds or one or more Dkk interacting or Dkk-1 interacting proteins, or a Dkk-binding fragment thereof. Other preferred Dkk modulating compositions comprise a Dkk or Dkk-1 antibody or an antibody to a Dkk interacting or Dkk-1 interacting protein. Also contemplated are antisense, siRNA, and shRNA molecules which recognize and bind to a nucleic acid encoding one or more Dkk-1 interacting proteins. The invention further provides such a method wherein the composition comprises a biologically active or LRP5/LRP6/HBM binding fragment of Dkk, including Dkk-1, such as those depicted in Figure 6 or a mimetic of those fragments depicted in Figure 6. The Dkk modulating composition may also comprise a peptide aptamer of a Dkk interacting or Dkk-1 interacting protein, or a mimetic of such an aptamer. A composition of the present invention may modulate activity either by enhancing or inhibiting the binding of Dkk, including Dkk-1, to LRP5, LRP6, or HBM or the binding of Dkk, including Dkk-1, to a Dkk interacting protein, such as those shown in Figure 5. The invention further provides such a method wherein the composition comprises an aptamer of Dkk or Dkk-1, such as those depicted. A composition of the present invention may comprise an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208). Preferred compositions of the present invention also comprise LRP5 antibodies.

A further aspect of the invention is to provide for a method of modulating Wnt signaling in a subject comprising administering a therapeutically effective amount of a composition which modulates Dkk activity or modulates Dkk interaction with LRP5

(or LRP6 or HBM). In a preferred embodiment, the Dkk protein is Dkk-1. In a particularly preferred embodiment, Dkk-1 activity is decreased. In another embodiment, Dkk activity modulates bone mass and/or lipid levels. In a preferred embodiment, bone mass is increased and/or lipid levels are decreased. In another preferred embodiment, the modulation in bone mass is an increase in bone strength determined via one or more of a decrease in fracture rate, an increase in areal bone density, an increase in volumetric mineral bone density, an increase in trabecular connectivity, an increase in trabecular density, an increase in cortical density or thickness, an increase in bone diameter, and an increase in inorganic bone content.

In another preferred embodiment, the Wnt is Wnt1-Wnt19. In a particularly preferred embodiment, the Wnt is Wnt1, Wnt3, Wnt3a, or Wnt10b. Preferred Wnt modulating compositions comprise one or more Dkk interacting or Dkk-1 interacting proteins, or a biologically active or LRP5/LRP6/HBM binding fragment thereof. Also contemplated are antisense, siRNA, and shRNA molecules which recognize and bind to a nucleic acid encoding one or more Dkk interacting or Dkk-1 interacting proteins. The invention further provides such a method wherein the composition comprises a biologically active or LRP5/LRP6/HBM binding fragment of Dkk or Dkk-1, such as those depicted in Figure 6 or a mimetic of those fragments depicted in Figure 6. The Dkk modulating composition may also comprise a peptide aptamer of a Dkk interacting or Dkk-1 interacting protein, or a mimetic of such an aptamer. A composition of the present invention may modulate activity either by enhancing or blocking the binding of Dkk, including Dkk-1, to LRP5, LRP6, or HBM or the binding of Dkk or Dkk-1 to a Dkk interacting or Dkk-1 interacting protein, such as those shown in Figure 5. The invention further provides such a method wherein compositions comprising an aptamer of Dkk or Dkk-1, such as those depicted in Figure 3 (SEQ ID NOs:171-188), or a mimetic of such an aptamer. The invention further provides such a method wherein the composition comprises a Dkk or Dkk-1 antibody or an antibody to a Dkk interacting or Dkk-1 interacting protein. The invention further provides such a method wherein compositions of an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208), Figures 4 (SEQ ID NO:189-192

(particularly peptide (SEQ ID NO:191) and Figure 13 (including SEQ ID NOs:204-214), or a mimetic of such an aptamer. Additional preferred compositions of the present invention also comprise LRP5 antibodies.

5 Additionally, the invention provides for a method of modulating bone mass and/or lipid levels in a subject comprising administering to the subject a composition which modulates Dkk activity or Dkk interaction with LRP5 in an amount effective to modulate bone mass and/or lipid levels, wherein bone mass and/or lipid levels are in need of modulation. In a preferred embodiment, the Dkk protein is Dkk-1. In a particularly preferred embodiment, Dkk-1 activity is decreased. In another 10 embodiment, Dkk activity modulates bone mass and/or lipid levels. In a preferred embodiment, bone mass is increased and/or lipid levels are decreased. In another preferred embodiment, the modulation in bone mass is an increase in bone strength determined via one or more of a decrease in fracture rate, an increase in areal bone density, an increase in volumetric mineral bone density, an increase in trabecular 15 connectivity, an increase in trabecular density, an increase in cortical density or thickness, an increase in bone diameter, and an increase in inorganic bone content. Preferred bone mass and/or lipid modulating compositions comprise one or more Dkk interacting or Dkk-1 interacting proteins, or a biologically active or LRP5/LRP6/HBM binding fragment thereof. Also contemplated are antisense, 20 siRNA, and shRNA molecules which recognize and bind to a nucleic acid encoding one or more Dkk interacting or Dkk-1 interacting proteins. The invention further provides such a method wherein the composition comprises a biologically active or LRP5/LRP6/HBM binding fragment of Dkk, including Dkk-1, such as those depicted in Figure 6 or a mimetic of those fragments depicted in Figure 6. The Dkk 25 modulating composition may also comprise a peptide aptamer of a Dkk interacting or Dkk-1 interacting protein, or a mimetic of such an aptamer. The invention further provides such a method wherein the composition comprises an aptamer of Dkk or Dkk-1, such as those depicted in Figure 3 (SEQ ID NOs:171-188), or a mimetic of such an aptamer. A composition of the present invention may modulate activity 30 either by enhancing or inhibiting the binding of Dkk, including Dkk-1, to LRP5, LRP6,

or HBM or the binding of Dkk, including Dkk-1, to a Dkk interacting protein, such as those shown in Figure 5. The invention further provides such a method wherein the composition comprises a Dkk or Dkk-1 antibody or an antibody to a Dkk interacting or Dkk-1 interacting protein. A composition of the present invention may comprise
5 an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208), Figures 4 (SEQ ID NOs:189-192 (particularly peptide 13 (SEQ ID NO:191)) and 13 (including SEQ ID NOs:204-214), or a mimetic of such an aptamer. Preferred compositions of the present invention also comprise LRP5 antibodies. It is a further aspect of the invention that such lipid-modulated diseases include a cardiac condition,
10 atherosclerosis, familial lipoprotein lipase deficiency, familial apoprotein CII deficiency, familial type 3 hyperlipoproteinemia, familial hypercholesterolemia, familial hypertriglyceridemia, multiple lipoprotein-type hyperlipidemia, elevated lipid levels due to dialysis and/or diabetes, and an elevated lipid level of unknown etiology.

15 Bone disorders contemplated for treatment and/or diagnosis by the methods and compositions disclosed herein include a bone development disorder, a bone fracture, age related loss of bone, a chondrodystrophy, a drug-induced bone disorder, high bone turnover, hypercalcemia, hyperostosis, osteogenesis imperfecta, osteomalacia, osteomyelitis, osteoporosis, Paget's disease, osteoarthritis, and
20 rickets.

It is a further object of the invention to provide a method of screening for compounds or compositions which modulates the interaction of Dkk with LRP5, LRP6, HBM, or a Dkk-binding fragment of LRP5, LRP6, or HBM comprising:

- 25 (a) exposing Dkk or a LRP5/LRP6/HBM binding fragment thereof to a compound; and
- (b) determining whether said compound binds to Dkk or the LRP5/LRP6/HBM binding fragment thereof.

In a preferred embodiment, the Dkk is Dkk-1. In a particularly preferred embodiment, the binding of Dkk-1 to LRP5/LRP6/HBM is decreased.

It is a further object of the invention to provide a method of screening compounds or compositions which modulate the interaction of DKK with LRP5, LRP6, HBM, or a DKK-binding fragment thereof comprising:

- (a) exposing DKK or a LRP5/LRP6/HBM binding fragment thereof to a compound; and,
- (b) determining whether said compound modulates the interaction of Dkk with LRP5, LRP6, or HBM, or the Dkk-binding fragment of LRP5/LRP6/HBM.

In a preferred embodiment, the Dkk is Dkk-1. In a particularly preferred embodiment, the interaction of Dkk-1 with LRP5/LRP6/HBM is decreased.

It is a further object of the invention to provide a method of screening for compounds or compositions which modulates the interaction of Dkk with LRP5, LRP6, HBM, or a Dkk-binding fragment of LRP5, LRP6, or HBM comprising:

- (a) exposing Dkk or a LRP5/LRP6/HBM binding fragment thereof to a compound;
- (b) determining whether said compound binds to Dkk or the LRP5/LRP6/HBM binding fragment thereof; and,
- (c) further determining whether said compound modulates the interaction of Dkk with LRP5, LRP6, or HBM, or the Dkk-binding fragment of LRP5/LRP6/HBM.

In preferred embodiments of such methods, Dkk or a biologically active fragment thereof is attached to a solid substrate. In an alternative embodiment of the invention, LRP5/LRP6/HBM, or a biologically active fragment thereof (such as the ligand binding domain), is exposed to the compound. Another aspect of the invention provides for compounds and compositions identified by the disclosed methods. A preferred embodiment of the invention provides that the compound screened in an afore-mentioned method is one or more proteins which interact with Dkk, particularly Dkk-1, as depicted in Figure 5, or a LRP5/LRP6/HBM-binding fragment thereof. Another preferred embodiment provides that the compound comprises a Dkk or Dkk-1 peptide aptamer, such as those depicted in Figure 3 (SEQ

ID NOs:171-188), or a mimetic of such aptamers. The compound may also comprise a peptide aptamer of a Dkk interacting or Dkk-1 interacting protein, or a mimetic of such an aptamer. The method further provides that the compound comprises a Dkk or Dkk-1 antibody or an antibody to a Dkk-1 interacting protein.

5 The invention further provides that the compound may comprise an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208), Figure 4 (SEQ ID NOs:189-192) (particularly peptide 13 (SEQ ID NO:191)) and Figure 13 (including SEQ ID NOs:204-214), or a mimetic of such an aptamer. Preferred compounds of the present invention also comprise LRP5 antibodies.

10 It is a further object of the invention to provide a method of screening for compounds or compositions which modulate the interaction of Dkk and a Dkk interacting protein comprising:

- (a) exposing a Dkk interacting proteins or a Dkk-binding fragment thereof to a compound; and,
- 15 (b) determining whether said compound binds to a Dkk interacting proteins or the Dkk-binding fragment thereof.

In a preferred embodiment, the Dkk is Dkk-1.

20 It is a further object of the invention to provide a method of screening for compounds or compositions which modulate the interaction of Dkk and a Dkk interacting protein comprising:

- (a) exposing Dkk interacting protein(s) or a Dkk-binding fragment thereof to a compounds; and,
- 25 (b) determining whether said compound modulates the interaction of Dkk and Dkk interacting proteins.

It is a further object of the invention to provide a method of screening for compounds or compositions which modulate the interaction of Dkk and a Dkk interacting protein comprising:

- 30 (a) exposing a Dkk interacting proteins or a Dkk-binding fragment thereof to a compound;

- (b) determining whether said compound binds to a Dkk interacting proteins or the Dkk-binding fragment thereof; and,
- (c) further determining whether said compound modulates the interaction of Dkk and Dkk interacting proteins.

In a preferred embodiment, Dkk is Dkk-1.

In preferred embodiments of such methods, the Dkk interacting proteins, particularly Dkk-1 interacting proteins, or a Dkk-binding fragment thereof are attached to a solid substrate. Another aspect of the invention provides for compounds and compositions identified by the disclosed methods. A preferred embodiment provides that the compound comprises a Dkk or Dkk-1 peptide aptamer, such as those depicted in Figure 3 (SEQ ID NOs:171-188), or a mimetic of such aptamers. The compound may also comprise a peptide aptamer of a Dkk interacting or Dkk-1 interacting protein, or a mimetic of such an aptamer. The compound may also comprise an antibody to a Dkk interacting or Dkk-1 interacting protein.

It is another object of the invention to provide for a composition for treating bone mass disorders comprising a LRP5/LRP6/HBM modulating compound and a pharmaceutically acceptable excipient and/or carrier therefor. Preferred LRP5 (or LRP6 or HBM) modulating compounds include Dkk or Dkk-1 or a LRP5/LRP6/HBM binding fragment thereof. Also contemplated are compounds which comprise monoclonal or polyclonal antibodies or immunologically active fragments thereof which bind to Dkk, including Dkk-1, and a pharmaceutically acceptable excipient and/or carrier. Another preferred embodiment provides that the modulating compound comprises one or more Dkk interacting or Dkk-1 interacting proteins, or a biologically active fragment thereof. Also contemplated are compounds which comprise monoclonal or polyclonal antibodies or immunologically active fragments thereof which bind to Dkk interacting or Dkk-1 interacting proteins, or a biologically active fragment thereof, and a pharmaceutically acceptable excipient and/or carrier.

Another preferred embodiment provides that the modulating compound comprises an antisense, siRNA, and shRNA molecule which recognizes and binds to a nucleic acid encoding a Dkk interacting or Dkk-1 interacting protein. Another preferred embodiment provides that the modulating compound comprises a Dkk or Dkk-1 peptide aptamer, a mimetic of a Dkk or Dkk-1 peptide aptamer, a peptide aptamer of a Dkk interacting or Dkk-1 interacting protein, or a mimetic of such an aptamer. Another embodiment provides that the compound comprises an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208), Figure 4 (SEQ ID NOs:189-192) (particularly peptide) and Figure 13 (including SEQ ID NOs:204-214), or a mimetic of such an aptamer. Preferred compounds of the present invention also comprise LRP5 antibodies.

It is a further object of the invention to provide a pharmaceutical composition for treating a Dkk-mediated disease or condition comprising a compound which modulates Dkk activity and a carrier therefor, including pharmaceutically acceptable excipients. Such compositions include those wherein the compound comprises an antisense, siRNA, and shRNA molecule or an antibody which binds to Dkk, including Dkk-1, and thereby prevents it from interacting with LRP5, LRP6, or HBM. Other such compositions include one or more of Dkk interacting or Dkk-1 interacting proteins, such as those depicted in Figure 5, or a Dkk-binding fragment thereof, or a monoclonal or polyclonal antibody, or immunologically active fragment thereof, which binds to a Dkk interacting or Dkk-1 interacting protein or Dkk-binding fragment thereof. Other contemplated compositions include antisense, siRNA, and shRNA molecules which recognize and bind to a nucleic acid encoding a Dkk interacting or Dkk-1 interacting protein. Further contemplated compositions include Dkk and Dkk-1 peptide aptamers, such as those depicted in Figure 3 (SEQ ID NOs:171-188), mimetics of such aptamers, a peptide aptamer of a Dkk interacting or Dkk-1 interacting protein, or a mimetic of such an aptamer. Other contemplated compositions comprise an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208), Figure 4 (SEQ ID NOs:189-192) (particularly peptide 13 (SEQ ID NO:191))

and Figure 13 (including SEQ ID NO:204-214), or a mimetic of such an aptamer. Other preferred compositions of the present invention comprise LRP5 antibodies.

A further object of the invention to provide for a method of modulating the expression of a nucleic acid encoding a Dkk interacting or Dkk-1 interacting protein in an organism, such as those shown in Figure 5, comprising the step of administering to the organism an effective amount of composition which modulates the expression of a nucleic acid encoding a Dkk-1 interacting protein. In a preferred embodiment, said composition comprises an antisense, siRNA, or shRNA molecule which recognizes and binds to a nucleic acid encoding a Dkk interacting or Dkk-1 interacting protein.

One aspect of the invention provides for a method of modulating at least one activity of Dkk or a Dkk-1 interacting protein comprising administering an effective amount of a composition which modulates at least one activity of Dkk or a Dkk-1 interacting protein. The invention provides for a composition comprising a Dkk interacting or Dkk-1 interacting protein, such as those shown in Figure 5, or a biologically active fragment thereof. Other agents contemplated for this method are antisense, siRNA, or shRNA molecules which recognize and bind to a nucleic acid encoding a Dkk interacting or Dkk-1 interacting protein. The method further provides that the composition comprises a Dkk or Dkk-1 antibody or an antibody to a Dkk interacting or Dkk-1 interacting protein. In another preferred embodiment, the composition comprises a Dkk or Dkk-1 peptide aptamer, a mimetic of a Dkk or Dkk-1 peptide aptamer, a peptide aptamer of a Dkk interacting or Dkk-1 interacting protein, or a mimetic of such an aptamer. The method provides that a composition of the present invention may comprise an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208), Figure 4 (SEQ ID NO:189-192) (particularly peptide including (SEQ ID NO:191)) and Figure including (SEQ ID NOs:204-214), or a mimetic of such an aptamer. Preferred compositions of the present invention also comprise LRP5 antibodies. In a further preferred embodiment, the modulated Dkk activity is lipid modulation or bone mass modulation.

In all of the testing/screening embodiments of the present invention discussed below to obtain compounds or compositions which ultimately impact LRP5/LRP6/HBM signaling, one skilled in the art will recognize that HBM can be used as a control in the absence of a test sample or compound. Further, the effect of a test sample of compound on Wnt signaling through the interaction of Dkk with LRP5/LRP6/HBM does not necessarily require a direct measurement of an association or interaction of Dkk and LRP5/LRP6/HBM. Other positive phenotypes/activities established by the High Bone Mass phenotype or by using HBM as a control.

One aspect of the invention provides for a method of identifying binding partners for a Dkk protein comprising the steps of:

- (a) exposing the Dkk protein(s) or a LRP5/LRP6 binding fragment thereof to a potential binding partner; and
- (b) determining if the potential binding partner binds to a Dkk protein or the LRP5/LRP6 binding fragment thereof.

In a preferred embodiment, the Dkk is Dkk-1.

Another aspect of the invention is to provide for a method of identifying a compound that effects Dkk-mediated activity comprising

- (a) providing a group of transgenic animals having (1) a regulatable one or more Dkk interacting protein genes, (2) a knock-out of one or more Dkk interacting protein genes, or (3) a knock-in of one or more Dkk interacting protein genes;
- (b) providing a second group of control animals respectively for the group of transgenic animals in step (a); and
- (c) exposing the transgenic animal group and the control animal group to a potential Dkk-modulating compound which modulates bone mass or lipid levels; and

- (d) comparing the transgenic animal group and the control animal group and determining the effect of the compound on bone mass or lipid levels in the transgenic animals as compared to the control animals.

In a preferred embodiment, the Dkk is Dkk-1.

It is another aspect of the invention to provide for a method for determining whether a compound modulates a Dkk interacting protein, said method comprising the steps of:

- (a) mixing the Dkk interacting protein or a Dkk-binding fragment thereof with the ligand binding domain of Dkk in the presence of said at least one compound;
- (b) measuring the amount of said binding domain of Dkk bound to said Dkk interacting protein or the Dkk-binding fragment thereof as compared to a control without said at least one compound; and
- (c) determining whether the compound reduces the amount of said binding domain of Dkk binding to said Dkk interacting protein or Dkk-binding fragment thereof.

In a preferred embodiment, the Dkk is Dkk-1.

In a preferred embodiment, the binding domain is attached to a solid substrate. The invention further provides for compounds identified by this method.

In a preferred embodiment, the invention provides that the Dkk interacting or Dkk-1 interacting protein is detected by antibodies. In another preferred embodiment, the solid substrate is a microarray. Another preferred embodiment provides that the ligand binding domain of Dkk and/or Dkk interacting protein is fused or conjugated to a peptide or protein. The invention also provides that the compounds include Dkk

and Dkk-1 peptide aptamers, mimetics of Dkk and Dkk-1 peptide aptamers, Dkk and Dkk-1 interacting proteins peptide aptamers, or mimetics of such aptamers.

An aspect of the invention provides a composition comprising one or more polypeptide sequences of one or more Dkk-1 interacting proteins, or a biologically active fragment thereof, one or more Dkk proteins, or a biologically active fragment thereof, or LRP5/LRP6/HBM polypeptide sequences or a biologically active fragment thereof (for example, the ligand binding domain) and a pharmaceutically acceptable excipient and/or carrier. Another aspect of the invention provides that the composition comprises a Dkk or Dkk-1 antibody or an antibody to a Dkk interacting or Dkk-1 interacting protein and a pharmaceutically acceptable excipient. A composition of the present invention may comprise an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208), Figure 4 (SEQ ID NOs:189-192) (particularly peptide 13 (SEQ ID NO:191)) and Figure 13 (including SEQ ID NOs:204-214), or a mimetic of such an aptamer. A composition of the present invention may comprise a Dkk peptide aptamer, for example as shown in Figure 3 (SEQ ID NOs:171-188). Preferred compositions of the present invention also comprise LRP5 antibodies.

Another aspect of the invention is to provide an antibody or immunologically active antibody fragment which recognizes and binds to a Dkk-1 amino acid sequence selected from the group consisting of: Asn34-His266 (SEQ ID NO:110), Asn34-Cys245 (SEQ ID NO:111), Asn34-Lys182 (SEQ ID NO:112), Cys97-His266 (SEQ ID NO:113), Val139-His266 (SEQ ID NO:114), Gly183-His266 (SEQ ID NO:115), Cys97-Cys245 (SEQ ID NO:116), or Val139-Cys245 (SEQ ID NO:117) of human Dkk-1. Additional antibodies may bind to any of the sequences depicted in Figures 3 (SEQ ID NOs:171-188) and Figure 4 (SEQ ID NOs:189-192). Another aspect of the invention is to provide for polyclonal antibodies to one or more amino acid sequences: Peptide 1 -GNKYQTIDNYQPYPYPC (SEQ ID NO:118), Peptide 2 -LDGYSRRTTLSSKMYHTKGQEG (SEQ ID NO:119), Peptide 3 -RIQKDHHQASNSSRLHTCQRH (SEQ ID NO:120), Peptide 4 -RGEIEETITESFGND (SEQ ID NO:121), and Peptide 5 -EIFQRCYCGEGLSCRIQKD (SEQ ID NO: 122).

It is a further object of the invention to provide a nucleic acid encoding a Dkk protein, e.g. Dkk-1, a Dkk interacting or Dkk-1 interacting protein aptamer, or an LRP5 aptamer comprising a nucleic acid encoding a scaffold protein in-frame with the activation domain of Gal4 or LexA that is in-frame with a nucleic acid which
5 encodes for a Dkk or Dkk-1 or Dkk interacting or Dkk-1 interacting protein amino acid sequence. Preferably the scaffold protein is thioredoxin (trxA), S1 nuclease from *Staphylococcus* or M13. Other preferable embodiments include Dkk-1 amino acid sequences selected from Figure 6.

It is yet a further object of the invention to provide a composition comprising a
10 polypeptide sequence of Figure 3 (SEQ ID NOs:171-188), Figure 4 (SEQ ID NO:189-192), or of Dkk-1 interacting proteins identified in Figure 5 and a pharmaceutically acceptable excipient and/or carrier.

Another aspect of the invention includes a method of detecting the modulatory
15 activity of a compound on the binding interaction of a first peptide and a second peptide of a peptide binding pair that bind through extracellular interaction in their natural environment, comprising:

- (i) culturing at least one eukaryotic cell, wherein the eukaryotic cell comprises;
 - a) a nucleotide sequence encoding a first heterologous
20 fusion protein comprising the first peptide or a segment thereof joined to a DNA binding domain of a transcriptional activation protein;
 - b) a nucleotide sequence encoding a second heterologous
25 fusion protein comprising the second peptide or a segment thereof joined to a transcriptional activation domain of a transcriptional activation protein;

wherein binding of the first peptide or segment thereof and the second peptide or segment thereof reconstitutes a transcriptional activation protein; and

c) a reporter element activated under positive transcriptional control of the reconstituted transcriptional activation protein, wherein expression of the reporter element produces a selected phenotype;

- 5 (ii) incubating a compound with the eukaryotic cell under conditions suitable to detect the selected phenotype; and
- (iii) detecting the ability of the compound to affect the binding interaction of the peptide binding pair by determining whether the compound affects the expression of the reporter element which produces the
- 10 selected phenotype;

wherein (1) said first peptide is a Dkk peptide and said second peptide is a peptide selected from LRP5, HBM, LRP6, and the Dkk-binding portion of LRP5/LRP6/HBM or (2) said first peptide is a Dkk-interacting protein or the Dkk-binding fragment thereof, and said second peptide is a Dkk peptide.

15 In one embodiment, the eukaryotic cell is a yeast cell. In a preferred embodiment, the yeast cell is *Saccharomyces*. In a particularly preferred embodiment, the *Saccharomyces* cell is *Saccharomyces cerevisiae*. The invention further provides that the compound may comprise a Dkk interacting or Dkk-1 interacting protein, or a biologically active fragment thereof. In one embodiment, the

20 Dkk interacting or Dkk-1 interacting protein, or a Dkk-binding fragment thereof, is added directly to the assay. In another embodiment, the Dkk interacting or Dkk-1 interacting protein, or a Dkk-binding fragment thereof, is recombinantly expressed by the eukaryotic cell in addition to the first and second peptides. In a preferred

25 embodiment the compound comprises a Dkk or Dkk-1 aptamer, a mimetic of a Dkk or Dkk-1 peptide aptamer, a Dkk interacting or Dkk-1 interacting protein aptamer, or a mimetic of a Dkk-1 interacting protein aptamer. Other preferred embodiments provide that the compound comprises an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208), Figure 4 (SEQ ID NOs:189-192) (particularly peptide 13 (SEQ ID NO:191) and Figure 13 (including SEQ ID NOs:204-214), or a mimetic of such an

30 aptamer. Alternatively, the present invention also provides that the compound may

comprise LRP5 antibodies or Dkk antibodies. In another embodiment, the yeast cell further comprises at least one endogenous nucleotide sequence selected from the group consisting of a nucleotide sequence encoding the DNA binding domain of a transcriptional activation protein, a nucleotide sequence encoding the transcriptional activation domain of a transcriptional activation protein, and a nucleotide sequence encoding the reporter element, wherein at least one of the endogenous nucleotide sequences is inactivated by mutation or deletion. In another embodiment, the peptide binding pair comprises a ligand and a receptor to which the ligand binds. In one embodiment, the transcriptional activation protein is Gal4, Gcn4, Hap1, Adr1, Swi5, Ste12, Mcm1, Yap1, Ace1, Ppr1, Arg81, Lac9, Qa1F, VP16, or a mammalian nuclear receptor. In another embodiment, at least one of the heterologous fusion proteins is expressed from an autonomously-replicating plasmid. In one embodiment, the DNA binding domain comprises a heterologous DNA-binding domain of a transcriptional activation protein. In a preferred embodiment, the DNA binding protein is selected from the group consisting of a mammalian steroid receptor and bacterial LexA protein. In another embodiment, the reporter element is selected from the group consisting of *lacZ*, a polynucleotide encoding luciferase, a polynucleotide encoding green fluorescent protein (GFP), and a polynucleotide encoding chloramphenicol acetyltransferase. In a particularly preferred embodiment, the reporter element is *lacZ*.

The invention further provides for a rescue screen for detecting the activity of a compound for modulating the binding interaction of a first peptide and a second peptide of a peptide binding pair, comprising:

- (i) culturing at least one yeast cell, wherein the yeast cell comprises;
 - a) a nucleotide sequence encoding a first heterologous fusion protein comprising the first peptide or a segment thereof joined to a DNA binding domain of a transcriptional activation protein;
 - b) a nucleotide sequence encoding a second heterologous

fusion protein comprising the second peptide or a segment thereof joined to a transcriptional activation domain of a transcriptional activation protein;

wherein binding of the first peptide or segment thereof and the second peptide or segment thereof reconstitutes a transcriptional activation protein; and

c) a reporter element activated under positive transcriptional control of the reconstituted transcriptional activation protein, wherein expression of the reporter gene prevents exhibition of a selected phenotype;

(ii) incubating a compound with the yeast cell under conditions suitable to detect the selected phenotype; and

(iii) detecting the ability of the compound to affect the binding interaction of the peptide binding pair by determining whether the compound affects the expression of the reporter element which prevents exhibition of the selected phenotype,

wherein said first peptide is a Dkk peptide and said second peptide is a peptide selected from LRP5, HBM, LRP6 and a Dkk-binding fragment of LRP5/LRP6/HBM.

In a preferred embodiment, the invention provides that the yeast cell is *Saccharomyces*. In a particularly preferred embodiment, the *Saccharomyces* cell is *Saccharomyces cerevisiae*. In one embodiment, the compound comprises one or more Dkk interacting or Dkk-1 interacting proteins, or a Dkk-binding fragment thereof. Compounds used in the present invention may comprise an LRP5 peptide aptamer, such as OST262 (SEQ ID NO:208), Figure 4 (SEQ ID NOs:189-192) (particularly peptide 13 (SEQ ID NO:191)) and Figure 13 (including SEQ ID NOs:204-214), or a mimetic of such an aptamer. Alternatively, the compound may comprise LRP5 antibodies or Dkk antibodies. In another embodiment, the yeast cell further comprises at least one endogenous nucleotide sequence selected from the group consisting of a nucleotide sequence encoding the DNA binding domain of a

transcriptional activation protein, a nucleotide sequence encoding the transcriptional activation domain of a transcriptional activation protein, and a nucleotide sequence encoding the reporter gene, wherein at least one of the endogenous nucleotide sequences is inactivated by mutation or deletion. In another embodiment, the transcriptional activation protein is Gal4, Gcn4, Hap1, Adr1, Swi5, Ste12, Mcm1, Yap1, Ace1, Ppr1, Arg81, Lac9, Qa1F, VP16, or a mammalian nuclear receptor. In one embodiment, at least one of the heterologous fusion proteins is expressed from an autonomously-replicating plasmid. In another embodiment, the DNA binding domain is a heterologous DNA-binding domain of a transcriptional activation protein.

The invention also provides for a rescue screen for detecting the modulatory activity of a compound on the binding interaction of a first peptide and a second peptide of a peptide binding pair, comprising:

- (i) culturing at least one yeast cell, wherein the yeast cell comprises;
 - a) a nucleotide sequence encoding a first heterologous fusion protein comprising the first peptide or a segment thereof joined to a DNA binding domain of a transcriptional activation protein;
 - b) a nucleotide sequence encoding a second heterologous fusion protein comprising the second peptide or a segment thereof joined to a transcriptional activation domain of a transcriptional activation protein;wherein binding of the first peptide or segment thereof and the second peptide or segment thereof reconstitutes a transcriptional activation protein; and
 - c) a reporter element activated under positive transcriptional control of the reconstituted transcriptional activation protein, wherein expression of the reporter element prevents exhibition of a selected phenotype;
- (ii) incubating a compound with the yeast cell under conditions suitable to detect the selected phenotype; and

- (iii) detecting the ability of the compound to affect the binding interaction of the peptide binding pair by determining whether the compound affects the expression of the reporter element which prevents exhibition of the selected phenotype,

5 wherein said first peptide is a Dkk interacting or Dkk-1 interacting protein peptide and said second peptide is a Dkk or Dkk-1 peptide.

In a preferred embodiment of the rescue screen, the yeast cell is *Saccharomyces*. In a particularly preferred embodiment, the *Saccharomyces* cell is *Saccharomyces cerevisiae*. In another embodiment, the yeast cell further comprises
10 at least one endogenous nucleotide sequence selected from the group consisting of a nucleotide sequence encoding the DNA binding domain of a transcriptional activation protein, a nucleotide sequence encoding the transcriptional activation domain of a transcriptional activation protein, and a nucleotide sequence encoding the reporter gene, wherein at least one of the endogenous nucleotide sequences is
15 inactivated by mutation or deletion. In one embodiment, the transcriptional activation protein is Gal4, Gcn4, Hap1, Adr1, Swi5, Ste12, Mcm1, Yap1, Ace1, Ppr1, Arg81, Lac9, Qa1F, VP16, or a mammalian nuclear receptor. In another embodiment of the rescue screen, at least one of the heterologous fusion proteins is expressed from an autonomously-replicating plasmid. In another embodiment, the DNA binding domain
20 is a heterologous DNA-binding domain of a transcriptional activation protein.

The invention also provides for a method for identifying potential compounds which modulate Dkk activity comprising:

- a) measuring the effect on binding of one or more Dkk interacting protein, or a Dkk-binding fragment thereof, with Dkk or a
25 LRP5/LRP6/HBM binding fragment thereof in the presence and absence of a compound; and
- b) identifying as a potential Dkk modulatory compound a compound which modulates the binding between one or more Dkk interacting proteins or Dkk-binding fragment thereof and
30 Dkk or LRP5/LRP6/HBM fragment thereof.

In a preferred embodiment, the Dkk is Dkk-1.

The invention further provides for any of the Dkk peptide aptamers of Figure 3 (SEQ ID NOs:171-188). The invention also provides for any of the LRP peptide aptamers of Figure 4 (SEQ ID NOs:189-192).

5 Another aspect of the invention provides for a method of identifying agents which modulate the interaction of Dkk with the Wnt signaling pathway comprising:

- (a) injecting mRNA encoding Dkk and an agent into a *Xenopus* blastomere;
- (b) assessing axis duplication or analyzing marker gene expression; and
- 10 (c) identifying agents which elicit changes in axis duplication or marker

gene expression as agents which modulate the interaction of Dkk with the Wnt signaling pathway. Wherein the agent may be chosen from among mRNA encoding Dkk interacting proteins, fragments thereof, siRNA, shRNA, antisense nucleotides, and antibodies. In a preferred embodiment, Dkk is Dkk-1. In a further embodiment, mRNA of HBM, LRP5/6, any Wnt (including Wnt1-Wnt19, particularly Wnt1, Wnt3, Wnt3a, and Wnt10b), Wnt antagonist, or combination of these is co-injected into the *Xenopus* blastomere. In another embodiment, the marker gene analyzed could include Siamois, Xnr3, slug, Xbra, HNK-1, endodermin, Xlhx8, BMP2, BMP4, XLRP6, EF-1, or ODC.

20 The present invention provides for a method for identifying agents which modulate the interaction of Dkk with the Wnt signaling pathway comprising:

- (a) transfecting cells with constructs encoding Dkk and potential Dkk interacting proteins, mRNA fragments thereof, siRNA, shRNA, or antisense, antibodies to LRP5/HBM/LRP6/Dkk/Dkk-interacting protein;
 - 25 (b) assessing changes in expression of a reporter gene linked to a Wnt-responsive promoter; and,
 - (c) identifying as a Dkk interacting protein any protein which alters reporter gene expression compared with cells transfected with a Dkk construct alone. In a further preferred embodiment, the cells may be HOB-03-CE6, HEK293, or U2OS cells.
- 30

In alternative embodiments, the Wnt-responsive promoter is TCF or LEF. In other preferred embodiments, the cells are co-transfected with CMV beta-galactosidase or tk-Renilla.

The present invention further provides for a LRP5/HBM monoclonal or polyclonal antibody to one or more peptides of amino acid sequences MYWTDWVETPRIE (SEQ ID NO:123), MYWTDWGETPRIE (SEQ ID NO:124), KRTGGKRKEILSA (SEQ ID NO:125), ERVEKTTGDKRTRIQGR (SEQ ID NO:126), or KQQCDSFPDCIDGSDE (SEQ ID NO:127).

Additionally, the present invention provides a method for identifying compounds which modulate Dkk and LRP5/LRP6/HBM interactions comprising:

- (a) immobilizing LRP5/LRP6/HBM to a solid surface; and
- (b) treating the solid surface with a secreted Dkk protein or a secreted epitope-tagged Dkk and a test compound; and
- (c) determining whether the compound regulates binding between Dkk and LRP5/LRP6/HBM using antibodies to Dkk or the epitope tag or by directly measuring activity of an epitope tag.

In one embodiment, the Dkk is Dkk-1. In a preferred embodiment, the epitope tag is alkaline phosphatase, histidine, myc, or a V5 tag.

Another embodiment of the present invention provides for a method for identifying compounds which modulate Dkk and LRP5/LRP6/HBM interactions comprising:

- (a) creating an LRP5, LRP6, or HBM fluorescent fusion protein using a first fluorescent tag;
- (b) creating a Dkk fusion protein comprising a second fluorescent tag;
- (c) adding a test compound; and,
- (d) assessing changes in the ratio of fluorescent tag emissions using Fluorescence Resonance Energy Transfer (FRET) or Bioluminescent Resonance Energy Transfer (BRET) to determine whether the compound modulates Dkk and LRP5/LRP6/HBM interactions.

In a preferred embodiment, the Dkk is Dkk-1.

The present invention also provides for a method of diagnosing low or high bone mass and/or low or high lipid levels in a subject comprising examining expression of Dkk, LRP5, LRP6, HBM or HBM-like variant in the subject and determining whether Dkk, LRP5, LRP6, or HBM or a HBM-like variant is over- or under-expressed to determine whether subject has (a) high or low bone mass and/or (b) high or low lipid levels.

The invention further provides for a transgenic animal wherein Dkk is knocked out in a tissue-specific fashion. In a preferred embodiment, the Dkk is Dkk-1. In one preferred embodiment, the tissue specificity is bone tissue. In another preferred embodiment, the tissue specificity is liver or other tissues or cells involved in regulating lipid metabolism or cancer tissue.

The present invention further provides a method of screening for compounds which modulate the interaction of Dkk with LRP5, LRP6, or HBM comprising:

- (a) exposing LRP5, LRP6, or HBM, or a Dkk-binding fragment of LRP5, LRP6, or HBM to a compound; and
- (b) determining whether said compound bound to LRP5, LRP6, or HBM or the Dkk-binding fragment of LRP5, LRP6, or HBM and further determining whether said compound modulates the interaction of Dkk and LRP5, LRP6, or HBM.

In one embodiment, the Dkk is Dkk-1. In a preferred embodiment, the compound comprises an LRP5 peptide aptamer. Other preferred compositions include the peptide aptamer, OST262 (SEQ ID NO:208), Figure 4 (SEQ ID NOs:189-192) (particularly peptide 13 (SEQ ID NO:191) and Figure 13 (including SEQ ID NOs:204-214), or a mimetic of such an aptamer, and an LRP5 antibody.

The present invention also provides a method for identifying compounds which modulate Dkk and LRP5/LRP6/HBM interactions comprising:

- (a) immobilizing LRP5/LRP6/HBM to a solid surface; and
- (b) treating the solid surface with a secreted Dkk protein or a secreted epitope-tagged Dkk and a test compound; and

- (c) determining whether the compound regulates binding between Dkk and LRP5/LRP6/HBM using antibodies to Dkk or the epitope tag or by directly measuring activity of an epitope tag. In a preferred embodiment, the epitope tag is alkaline phosphatase, histidine, myc or a V5 tag.

In a preferred embodiment, the Dkk is Dkk-1.

The invention also provides for a method for identifying compounds which modulate the interaction of Dkk with the Wnt signaling pathway comprising:

- (a) transfecting cells with constructs containing Dkk and Wnt proteins;
(b) assessing changes in expression of a reporter element linked to a Wnt-responsive promoter; and
(c) identifying as a Dkk/Wnt interaction modulating compound any compound which alters reporter gene expression compared with cells transfected with a Dkk construct alone.

In one embodiment, the Dkk is Dkk-1. In another embodiment, the Wnt is any of Wnt1-Wnt19. In a preferred embodiment, the Wnt is Wnt1, Wnt3, Wnt3a, or Wnt10b. In a particularly preferred embodiment, the Wnt construct contains Wnt3a. In another particularly preferred embodiment, the Wnt construct contains Wnt1. In another preferred embodiment, the Wnt construct encodes for a Wnt that signals through the canonical Wnt pathway. In a particularly preferred embodiment, both Wnt3a and Wnt1 constructs are co-transfected into the cells. In another embodiment, the cells may be U2-OS, HOB-03-CE6, or HEK293 cells. In another embodiment, the reporter element used is TCF-luciferase, tk-Renilla, or a combination thereof.

The invention also provides for a method of testing compounds that modulate Dkk-mediated activity in a mammal comprising:

- (a) providing a group of transgenic animals having (1) a regulatable one or more Dkk genes, (2) a knock-out of Dkk genes, or (3) a knock-in of one or more Dkk genes;

- (b) providing a second group of control animals respectively for the group of transgenic animals in step (a); and
- (c) exposing the transgenic animal group and control animal group to a potential Dkk-modulating compound which modulates bone mass or lipid levels; and
- (d) comparing the transgenic animals and the control group of animals and determining the effect of the compound on bone mass or lipid levels in the transgenic animals as compared to the control animals.

In a preferred embodiment, the Dkk is Dkk-1.

The invention further provides variants of LRP5 which demonstrate HBM biological activity, i.e., that are "HBM-like." In preferred embodiments, variants G171F, M282V, G171K, G171Q, A65V, G171V, G171I, and A214V of LRP5 are provided. The invention further provides for the use any of these variants in the foregoing methods.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a schematic of the components of the Wnt signal transduction pathway. Schematic obtained from:

<http://www.stanford.edu/~rnusse/pathways/cell2.html>

Figure 2 (A-C) show bait sequences (SEQ ID NOs:168-170) utilized in yeast two hybrid (Y2H) screens for protein-protein interactions.

Figure 3 shows a table of peptide aptamer insert sequences (SEQ ID NOs: 171-192) identified in Y2H screen with a Dkk-1 bait sequence.

Figure 4 shows a table of peptide aptamer insert sequences identified in a Y2H screen using a LRP5 ligand binding domain bait sequence.

Figure 5 shows a table of proteins identified in a Y2H screen using a Dkk-1 bait sequence. These proteins are identified by both their nucleic acid and amino acid accession numbers.

Figure 6 shows the results of a minimum interaction domain mapping screen of Dkk-1 with LRP5. At the top, a map of Dkk-1 showing the location of the signal

sequence, and cysteine rich domains 1 and 2. Below, the extent of domains examined using LRP5 LBD baits, LBD1 and LBD4, of Figure 2. To the right, scoring of the binding results observed in the experiment.

Figure 7 shows a diagram of the *Xenopus* Embryo Assay for Wnt activity.

5 Figure 8 shows the effects of Zmax/LRP5 and HBM on Wnt signaling in the *Xenopus* embryo assay.

Figure 9 shows the effects of Zmax/LRP5 and HBM on induction of secondary axis formation in the *Xenopus* embryo assay.

10 Figure 10 shows the effects of human Dkk-1 on the repression of the canonical Wnt pathway.

Figure 11 shows the effects of human Dkk-1 on Zmax/LRP5 and HBM-mediated Wnt signaling.

15 Figure 12 shows pcDNA3.1 construct names with nucleotide sequences (including SEQ ID NOs:193-203) for LRP5-binding peptide aptamers, Dkk-1 peptides and control constructs.

Figure 13 shows the amino acid sequences (including SEQ ID NOs:204-214) for the corresponding LRP5-binding peptides, Dkk-1 peptide aptamers and control constructs in Figure 12.

20 Figure 14 shows the effects of Dkk-1 and Dkk-2 on Wnt1 signaling with coreceptors LRP5, HBM, and LRP6 in HOB03CE6 cells.

Figure 15 shows the effects of Dkk-1 and Dkk-2 on Wnt3a signaling with coreceptors LRP5, HBM, and LRP6 in HOB03CE6 cells.

Figure 16 demonstrates that the LRP5-LBD peptide aptamer 262 activates Wnt signaling in the presence of Wnt3a in U2OS cells.

25 Figure 17 shows the differential binding of an antibody generated to a sequence (a.a. 165-177) containing the HBM mutation in LRP5 in LRP5 and HBM virus-infected cells.

30 Figure 18 shows data generated from a Y2H interaction trap where a mutant Dkk-1 (C220A) is unable to bind to LRP5 and demonstrating the window of capability of detecting small molecule effects on LRP and Dkk interactions.

Figure 19 shows that Dkk-1 represses Wnt3a-mediated Wnt signaling in U2OS bone cells using the cell-based reporter gene assay for high throughput screening.

Figure 20 demonstrates that Wnt1-HBM generated signaling is not efficiently inhibited by Dkk-1 in U2OS bone cells while LRP5 and LRP6-mediated signaling are using the cell-based reporter gene assay for high throughput screening.

Figure 21 shows that the TCF signal in the cell-based reporter gene assay for high throughput screening can be modulated by Dkk-1 and Dkk-1-AP without Wnt DNA transfection.

Figure 22 shows the morphological results in the Xenopus assay using aptamers 261 and 262 from the LRP5-LBD to activate Wnt signaling.

Figure 23 demonstrates that LRP5-LBD aptamers 261 and 262 induce Wnt signaling over other LRP5 aptamers.

Figure 24 shows that the mutation G171F in LRP5 produces a greater activation of the Wnt pathway than LRP5 which is consistent with HBM activity.

Figure 25 shows that the mutation M282V in LRP5 produces an activation of the Wnt pathway which is consistent with HBM activity in U2OS cells.

Figure 26 shows the amino acid sequence of the various peptides of dkk-1 selected to generate polyclonal antibodies, their relationship to the Dkk-1 amino acid sequence and identities of polyclonal antibodies generated.

Figure 27 shows a Western blot demonstrating that polyclonal antibody #5521 to amino acids 165-186 of Dkk-1 was able to detect Dkk1-V5 and Dkk1-AP from conditioned medium.

Figure 28 shows a Western blot demonstrating that polyclonal antibody #74397 to amino acids 147-161 was able to detect Dkk1-V5 in both conditioned medium and immunoprecipitated conditioned medium.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

In general, terms in the present application are used consistent with the manner in which those terms are understood in the art. To aid in the understanding of the specification and claims, the following definitions are provided.

"Gene" refers to a DNA sequence that encodes through its template or messenger RNA a sequence of amino acids characteristic of a specific peptide. The term "gene" includes intervening, non-coding regions, as well as regulatory regions, and can include 5' and 3' ends.

By "nucleic acid" is meant to include single stranded and double stranded nucleic acids including, but not limited to DNAs, RNAs (e.g., mRNA, tRNAs, siRNAs), cDNAs, recombinant DNA (rDNA), rRNAs, antisense nucleic acids, oligonucleotides, and oligomers, and polynucleotides. The term may also include hybrids such as triple stranded regions of RNA and/or DNA or double stranded RNA:DNA hybrids. The term also is contemplated to include modified nucleic acids such as, but not limited to biotinylated nucleic acids, tritylated nucleic acids, fluorophor labeled nucleic acids, inosine, and the like.

"Gene sequence" refers to a nucleic acid molecule, including DNA which contains a non-transcribed or non-translated sequence, which comprises a gene. The term is also intended to include any combination of gene(s), gene fragment(s), non-transcribed sequence(s) or non-translated sequence(s) which are present on the same DNA molecule.

The nucleic acid sequences of the present invention may be derived from a variety of sources including DNA, cDNA, synthetic DNA, synthetic RNA or combinations thereof. Such sequences may comprise genomic DNA which may or may not include naturally occurring introns. Moreover, such genomic DNA may be obtained in association with promoter regions and/or poly (A) sequences. The sequences, genomic DNA or cDNA may be obtained in any of several ways. Genomic DNA can be extracted and purified from suitable cells by means well

known in the art. Alternatively, mRNA can be isolated from a cell and used to produce cDNA by reverse transcription or other means.

"cDNA" refers to complementary or copy DNA produced from an RNA template by the action of RNA-dependent DNA polymerase (reverse transcriptase).

5 Thus, a "cDNA clone" means a duplex DNA sequence for which one strand is complementary to an RNA molecule of interest, carried in a cloning vector or PCR amplified. cDNA can also be single stranded after first strand synthesis by reverse transcriptase. In this form, it is a useful PCR template and does not need to be carried in a cloning vector. This term includes genes from which the intervening
10 sequences have been removed. Thus, the term "gene", as sometimes used generically, can also include nucleic acid molecules comprising cDNA and cDNA clones.

"Recombinant DNA" means a molecule that has been engineered by splicing
15 *in vitro* a cDNA or genomic DNA sequence or altering a sequence by methods such as PCR mutagenesis.

"Cloning" refers to the use of *in vitro* recombination techniques to insert a particular gene or other DNA sequence into a vector molecule. In order to successfully clone a desired gene, it is necessary to use methods for generating DNA fragments, for joining the fragments to vector molecules, for introducing the
20 composite DNA molecule into a host cell in which it can replicate, and for selecting the clone having the target gene from amongst the recipient host cells.

"cDNA library" refers to a collection of recombinant DNA molecules containing cDNA inserts which together comprise the entire or a partial repertoire of genes expressed in a particular tissue or cell source. Such a cDNA library can be prepared
25 by methods known to one skilled in the art and described by, for example, Cowell and Austin, "cDNA Library Protocols," *Methods in Molecular Biology* (1997).

"Cloning vehicle" refers to a plasmid or phage DNA or other DNA sequence which is able to replicate in a host cell. This term can also include artificial chromosomes such as BACs and YACs. The cloning vehicle is characterized by one
30 or more endonuclease recognition sites at which such DNA sequences may be cut in

a determinable fashion without loss of an essential biological function of the DNA, which may contain a marker suitable for use in the identification of transformed cells.

"Expression" refers to the process comprising transcription of a gene sequence and subsequent processing steps, such as translation of a resultant mRNA to produce the final end product of a gene. The end product may be a protein (such as an enzyme or receptor) or a nucleic acid (such as a tRNA, antisense RNA, or other regulatory factor). The term "expression control sequence" refers to a sequence of nucleotides that control or regulate expression of structural genes when operably linked to those genes. These include, for example, the lac systems, the trp system, major operator and promoter regions of the phage lambda, the control region of fd coat protein and other sequences known to control the expression of genes in prokaryotic or eukaryotic cells. Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host, and may contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements and/or translational initiation and termination sites.

"Expression vehicle" refers to a vehicle or vector similar to a cloning vehicle but which is capable of expressing a gene which has been cloned into it, after transformation into a host. The cloned gene is usually placed under the control of (i.e., operably linked to) an expression control sequence.

"Operator" refers to a DNA sequence capable of interacting with the specific repressor, thereby controlling the transcription of adjacent gene(s).

"Promoter" refers to a DNA sequence that can be recognized by an RNA polymerase. The presence of such a sequence permits the RNA polymerase to bind and initiate transcription of operably linked gene sequences.

"Promoter region" is intended to include the promoter as well as other gene sequences which may be necessary for the initiation of transcription. The presence of a promoter region is sufficient to cause the expression of an operably linked gene sequence. The term "promoter" is sometimes used in the art to generically indicate a promoter region. Many different promoters are known in the art which direct

expression of a gene in a certain cell types. Tissue-specific promoters can comprise nucleic acid sequences which cause a greater (or decreased) level of expression in cells of a certain tissue type.

"Operably linked" means that the promoter controls the initiation of expression of the gene. A promoter is operably linked to a sequence of proximal DNA if upon introduction into a host cell the promoter determines the transcription of the proximal DNA sequence(s) into one or more species of RNA. A promoter is operably linked to a DNA sequence if the promoter is capable of initiating transcription of that DNA sequence.

"Prokaryote" refers to all organisms without a true nucleus, including bacteria.

"Eukaryote" refers to organisms and cells that have a true nucleus, including mammalian cells.

"Host" includes prokaryotes and eukaryotes, such as yeast and filamentous fungi, as well as plant and animal cells. The term includes an organism or cell that is the recipient of a replicable expression vehicle.

The term "animal" is used herein to include all vertebrate animals, except humans. It also includes an individual animal in all stages of development, including embryonic and fetal stages. Preferred animals include higher eukaryotes such as avians, rodents (e.g., mice, rabbits, rats, chinchillas, guinea pigs, hamsters and the like), and mammals. Preferred mammals include bovine, equine, feline, canine, ovine, caprine, porcine, buffalo, humans, and primates.

A "transgenic animal" is an animal containing one or more cells bearing genetic information received, directly or indirectly, by deliberate genetic manipulation or by inheritance from a manipulated progenitor at a subcellular level, such as by microinjection or infection with a recombinant viral vector (e.g., adenovirus, retrovirus, herpes virus, adeno-associated virus, lentivirus). This introduced DNA molecule may be integrated within a chromosome, or it may be extra-chromosomally replicating DNA.

"Embryonic stem cells" or "ES cells" as used herein are cells or cell lines usually derived from embryos which are pluripotent meaning that they are un-

differentiated cells. These cells are also capable of incorporating exogenous DNA by homologous recombination and subsequently developing into any tissue in the body when incorporated into a host embryo. It is possible to isolate pluripotent cells from sources other than embryonic tissue by methods which are well understood in the art.

Embryonic stem cells in mice have enabled researchers to select for transgenic cells and perform gene targeting. This allows more genetic engineering than is possible with other transgenic techniques. For example, mouse ES cells are relatively easy to grow as colonies *in vitro*. The cells can be transfected by standard procedures and transgenic cells clonally selected by antibiotic resistance. See, for example, Doetschman *et al.*, 1994, *Gene transfer in embryonic stem cells*. In Pinkert (Ed.) Transgenic Animal Technology: A Laboratory Handbook. Academic Press Inc., New York, pp.115-146. Furthermore, the efficiency of this process is such that sufficient transgenic colonies (hundreds to thousands) can be produced to allow a second selection for homologous recombinants. Mouse ES cells can then be combined with a normal host embryo and, because they retain their potency, can develop into all the tissues in the resulting chimeric animal, including the germ cells. The transgenic modification can then be transmitted to subsequent generations.

Methods for deriving embryonic stem (ES) cell lines *in vitro* from early preimplantation mouse embryos are well known. See for example, Evans *et al.*, 1981 *Nature* 29: 154-6 and Martin, 1981, *Proc. Nat. Acad. Sci. USA*, 78: 7634-8. ES cells can be passaged in an undifferentiated state, provided that a feeder layer of fibroblast cells or a differentiation inhibiting source is present.

The term "somatic cell" indicates any animal or human cell which is not a sperm or egg cell or is capable of becoming a sperm or egg cell. The term "germ cell" or "germ-line cell" refers to any cell which is either a sperm or egg cell or is capable of developing into a sperm or egg cell and can therefore pass its genetic information to offspring. The term "germ cell-line transgenic animal" refers to a transgenic animal in which the genetic information was incorporated in a germ line cell, thereby conferring the ability to transfer the information to offspring. If such

offspring in fact possess some or all of that information, then they, too, are transgenic animals.

The genetic alteration of genetic information may be foreign to the species of animal to which the recipient belongs, or foreign only to the particular individual recipient. In the last case, the altered or introduced gene may be expressed differently than the native gene.

"Fragment" of a gene refers to any portion of a gene sequence. A "biologically active fragment" refers to any portion of the gene that retains at least one biological activity of that gene. For example, the fragment can perhaps hybridize to its cognate sequence or is capable of being translated into a polypeptide fragment encoded by the gene from which it is derived.

"Variant" refers to a gene that is substantially similar in structure and biological activity or immunological characteristics to either the entire gene or to a fragment of the gene. Provided that the two genes possess a similar activity, they are considered variant as that term is used herein even if the sequence of encoded amino acid residues is not identical. Preferentially, as used herein (unless otherwise defined) the variant is one of LRP5, HBM or LRP6. The variant preferably is one that yields an HBM-like phenotype (i.e., enhances bones mass and/or modulates lipid levels). These variants include missense mutations, single nucleotide polymorphisms (SNPs), mutations which result in changes in the amino acid sequence of the protein encoded by the gene or nucleic acid, and combinations thereof, as well as com in the exon domains of the *HBM* gene and mutations in LRP5 or LRP6 which result in an HBM like phenotype.

"Amplification of nucleic acids" refers to methods such as polymerase chain reaction (PCR), ligation amplification (or ligase chain reaction, LCR) and amplification methods based on the use of Q-beta replicase. These methods are well known in the art and described, for example, in U.S. Patent Nos. 4,683,195 and 4,683,202. Reagents and hardware for conducting PCR are commercially available. Primers useful for amplifying sequences from the HBM region are preferably complementary to, and hybridize specifically to sequences in the HBM region or in

regions that flank a target region therein. HBM sequences generated by amplification may be sequenced directly. Alternatively, the amplified sequence(s) may be cloned prior to sequence analysis.

"Antibodies" may refer to polyclonal and/or monoclonal antibodies and fragments thereof, and immunologic binding equivalents thereof, that can bind to the HBM proteins and fragments thereof or to nucleic acid sequences from the HBM region, particularly from the HBM locus or a portion thereof. Preferred antibodies also include those capable of binding to LRP5, LRP6 and HBM variants. The term antibody is used both to refer to a homogeneous molecular entity, or a mixture such as a serum product made up of a plurality of different molecular entities. Proteins may be prepared synthetically in a protein synthesizer and coupled to a carrier molecule and injected over several months into rabbits. Rabbit sera is tested for immunoreactivity to the HBM protein or fragment. Monoclonal antibodies may be made by injecting mice with the proteins, or fragments thereof. Monoclonal antibodies will be screened by ELISA and tested for specific immunoreactivity with HBM protein or fragments thereof. Harlow *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988) and *Using Antibodies: A Laboratory Manual*, Harlow, Ed and Lane, David (Cold Spring Harbor Press, 1999). These antibodies will be useful in assays as well as pharmaceuticals. By "antibody" is meant to include but not limited to polyclonal, monoclonal, chimeric, human, humanized, bispecific, multispecific, primatized™ antibodies.

"HBM protein" refers to a protein that is identical to a Zmax1 (LRP5) protein except that it contains an alteration of glycine 171 to a valine. An HBM protein is defined for any organism that encodes a Zmax1 (LRP5) true homolog. For example, a mouse HBM protein refers to the mouse Zmax1 (LRP5) protein having the glycine 170 to valine substitution.

By "HBM-like" is meant a variant of LRP5, LRP6 or HBM which when expressed in a cell is capable of modulating bone mass, lipid levels, Dkk activity, and/or Wnt activity.

In one embodiment of the present invention, "*HBM* gene" refers to the genomic DNA sequence found in individuals showing the HBM characteristic or phenotype, where the sequence encodes the protein indicated by SEQ ID NO: 4. The *HBM* gene and the *Zmax1* (*LRP5*) gene are allelic. The protein encoded by the *HBM* gene has the property of causing elevated bone mass, while the protein encoded by the *Zmax1* (*LRP5*) gene does not. The *HBM* gene and the *Zmax1* (*LRP5*) gene differ in that the *HBM* gene has a thymine at position 582, while the *Zmax1* gene has a guanine at position 582. The *HBM* gene comprises the nucleic acid sequence shown as SEQ ID NO: 2. The *HBM* gene may also be referred to as an "HBM polymorphism." Other *HBM* genes may further have silent mutations, such as those discussed in Section 3 below.

In alternative embodiments of the present invention, "HBM gene" may also refer to any allelic variant of *Zmax1* (*LRP5*) or *LRP6* which results in the HBM phenotype. Such variants may include alteration from the wild-type protein coding sequence as described herein and/or alteration in expression control sequences of *Zmax1* (*LRP5*) or contains an amino acid mutation in *LRP5* or *LRP6*, such that the resulting protein produces a phenotype which enhances bone mass and/or modulates lipid levels. A preferred example of such a variant is an alteration of the endogenous *Zmax1* (*LRP5*) promoter region resulting in increased expression of the *Zmax1* (*LRP5*) protein.

"Normal," "wild-type," "unaffected", "*Zmax1*", "*Zmax*", "*LR3*" and "*LRP5*" all refer to the genomic DNA sequence that encodes the protein indicated by SEQ ID NO: 3. *LRP5* has also been referred to *LRP7* in mouse. *Zmax1*, *LRP5* and *Zmax* may be used interchangeably throughout the specification and are meant to be the same gene, perhaps only relating to the gene in a different organism. The *Zmax1* gene has a guanine at position 582 in the human sequence. The *Zmax1* gene of human comprises the nucleic acid sequence shown as SEQ ID NO: 1. "Normal," "wild-type," "unaffected", "*Zmax1*" and "*LRP5*" also refer to allelic variants of the genomic sequence that encodes proteins that do not contribute to elevated bone

mass. The *Zmax1* (*LRP5*) gene is common in the human population, while the *HBM* gene is rare.

"Bone development" generally refers to any process involved in the change of bone over time, including, for example, normal development, changes that occur during disease states, and changes that occur during aging. This may refer to structural changes and dynamic rate changes such as growth rates, resorption rates, bone repair rates, and etc. "Bone development disorder" particularly refers to any disorders in bone development including, for example, changes that occur during disease states and changes that occur during aging. Bone development may be progressive or cyclical in nature. Aspects of bone that may change during development include, for example, mineralization, formation of specific anatomical features, and relative or absolute numbers of various cell types.

"Bone modulation" or "modulation of bone formation" refers to the ability to affect any of the physiological processes involved in bone remodeling, as will be appreciated by one skilled in the art, including, for example, bone resorption and appositional bone growth, by, *inter alia*, osteoclastic and osteoblastic activity, and may comprise some or all of bone formation and development as used herein.

Bone is a dynamic tissue that is continually adapting and renewing itself through the renewal of old or unnecessary bone by osteoclasts and the rebuilding of new bone by osteoblasts. The nature of the coupling between these processes is responsible for both the modeling of bone during growth as well as the maintenance of adult skeletal integrity through remodeling and repair to meet the everyday needs of mechanical usage. There are a number of diseases that result from an uncoupling of the balance between bone resorption and formation. With aging there is a gradual "physiologic" imbalance in bone turnover, which is particularly exacerbated in women due to menopausal loss of estrogen support, that leads to a progressive loss of bone. As bone mineral density falls below population norms there is a consequent increase in bone fragility and susceptibility to spontaneous fractures. For every 10 percent of bone that is lost, the risk of fracture doubles. Individuals with bone mineral density (BMD) in the spine or proximal femur 2.5 or

more standard deviations below normal peak bone mass are classified as osteoporotic. However, osteopenic individuals with BMD between 1 and 2.5 standard deviations below the norm are clearly at risk.

Bone is measured by several different forms of X-ray absorptiometry. All of the instruments measure the inorganic or bone mineral content of the bone. Standard DXA measurements give a value that is an areal density, not a true density measurement by the classical definition of density (mass/unit volume). Nevertheless, this is the type of measurement used clinically to diagnose osteoporosis. However, while BMD is a major contributing factor to bone strength, as much as 40% of bone strength stems from other factors including: 1) bone size (*i.e.*, larger diameters increase organ-level stiffness, even in the face of lower density); 2) the connectivity of trabecular structures; 3) the level of remodeling (remodeling loci are local concentrators of strain); and 4) the intrinsic strength of the bony material itself, which in turn is a function of loading history (*i.e.*, through accumulated fatigue damage) and the extent of collagen cross-linking and level of mineralization. There is good evidence that all of these strength/fragility factors play some role in osteoporotic fractures, as do a host of extraskkeletal influences as well (such as fall patterns, soft tissue padding, and central nervous system reflex responsiveness).

Additional analytical instruments can be used to address these features of bone. For example, the pQCT allows measurement of separate trabecular and cortical compartments for size and density and the μ CT provides quantitative information on architectural features such as trabecular connectivity. The μ CT also gives a true bone density measurement. With these tools, the important non-BMD parameters can be measured for diagnosing the extent of disease and the efficacy of treatments. Current treatments for osteoporosis are based on the ability of drugs to prevent or retard bone resorption. Although newer anti-resorptive agents are proving to be useful in the therapy of osteoporosis, they are viewed as short-term solutions to the more definitive challenge to develop treatments that will increase bone mass and/or the bone quality parameters mentioned above.

Thus, bone modulation may be assessed by measuring parameters such as bone mineral density (BMD) and bone mineral content (BMC) by pDXA X-ray methods, bone size, thickness or volume as measured by X-ray, bone formation rates as measured for example by calcein labeling, total, trabecular, and mid-shaft density as measured by pQCT and/or μ CT methods, connectivity and other histological parameters as measured by μ CT methods, mechanical bending and compressive strengths as preferably measured in femur and vertebrae respectively. Due to the nature of these measurements, each may be more or less appropriate for a given situation as the skilled practitioner will appreciate. Furthermore, parameters and methodologies such as a clinical history of freedom from fracture, bone shape, bone morphology, connectivity, normal histology, fracture repair rates, and other bone quality parameters are known and used in the art. Most preferably, bone quality may be assessed by the compressive strength of vertebra when such a measurement is appropriate. Bone modulation may also be assessed by rates of change in the various parameters. Most preferably, bone modulation is assessed at more than one age.

"Normal bone density" refers to a bone density within two standard deviations of a Z score of 0 in the context of the HBM linkage study. In a general context, the range of normal bone density parameters is determined by routine statistical methods. A normal parameter is within about 1 or 2 standard deviations of the age and sex normalized parameter, preferably about 2 standard deviations. A statistical measure of meaningfulness is the P value which can represent the likelihood that the associated measurement is significantly different from the mean. Significant P values are $P < 0.05$, 0.01 , 0.005 , and 0.001 , preferably at least $P < 0.01$.

"HBM" refers to "high bone mass" although this term may also be expressed in terms of bone density, mineral content, and size.

The "HBM phenotype" and "HBM-like phenotype" may be characterized by an increase of about 2 or more standard deviations, preferably 2, 2.5, 3, or more standard deviations in 1, 2, 3, 4, 5, or more quantitative parameters of bone modulation, preferably bone density and mineral content and bone strength

parameters, above the age and sex norm for that parameter. The HBM phenotype and HBM-like phenotype are characterized by statistically significant increases in at least one parameter, preferably at least 2 parameters, and more preferably at least 3 or more parameters. The HBM phenotype and the HBM-like phenotype may also be characterized by an increase in one or more bone quality parameters and most preferably increasing parameters are not accompanied by a decrease in any bone quality parameters. Most preferably, an increase in bone modulation parameters and/or bone quality measurements is observed at more than one age. The HBM phenotype and HBM-like phenotype also includes changes of lipid levels, Wnt activity and/or Dkk activity.

The terms "isolated" and "purified" refer to a substance altered by hand of man from the natural environment. An isolated peptide may be for example in a substantially pure form or otherwise displaced from its native environment such as by expression in an isolated cell line or transgenic animal. An isolated sequence may for example be a molecule in substantially pure form or displaced from its native environment such that at least one end of said isolated sequence is not contiguous with the sequence it would be contiguous with in nature.

"Biologically active" refers to those forms of proteins and polypeptides, including conservatively substituted variants, alleles of genes encoding a protein or polypeptide fragments of proteins which retain a biological and/or immunological activity of the wild-type protein or polypeptide. Preferably the activity is one which induces a change in Dkk activity, such as inhibiting the interaction of Dkk with a ligand binding partner (e.g., LRP5 or LRP6 or Dkk-1 with a Dkk-1 interacting protein such as those shown in Figure 5). By biologically active is also meant to include any form which modulates Wnt signaling.

By "modulate" and "regulate" is meant methods, conditions, or agents which increase or decrease the wild-type activity of an enzyme, inhibitor, signal transducer, receptor, transcription activator, co-factor, and the like. This change in activity can be an increase or decrease of mRNA translation, mRNA or DNA transcription, and/or

mRNA or protein degradation, which may in turn correspond to an increase or decrease in biological activity.

By "modulated activity" is meant any activity, condition, disease or phenotype which is modulated by a biologically active form of a protein. Modulation may be effected by affecting the concentration or subcellular localization of biologically active protein, *i.e.*, by regulating expression or degradation, or by direct agonistic or antagonistic effect as, for example, through inhibition, activation, binding, or release of substrate, modification either chemically or structurally, or by direct or indirect interaction which may involve additional factors.

By "effective amount" or "dose effective amount" or "therapeutically effective amount" is meant an amount of an agent which modulates a biological activity of the polypeptide of the invention.

By "immunologically active" is meant any immunoglobulin protein or fragment thereof which recognizes and binds to an antigen.

By "Dkk" is meant to refer to the nucleic acids and proteins of members of the Dkk (Dickkopf) family. This includes, but is not limited to, Dkk-1, Dkk-2, Dkk-3, Dkk-4, Soggy, and related Dkk proteins. Dkk-1 is a preferred embodiment of the present invention. However, the Dkk proteins have substantial homology and one skilled in the art will appreciate that all of the embodiments of the present invention utilizing Dkk-1 may also be utilized with the other Dkk proteins.

By "Dkk-1" is meant to refer to the Dkk-1 protein and nucleic acids which encode the Dkk-1 protein. Dkk-1 refers to Dickkopf-1, and in *Xenopus* it is related to at least Dkk-2, Dkk-3, and Dkk-4 (see Krupnik *et al.*, *Gene* 238:301-313 (1999)). Dkk-1 was first identified in *Xenopus* (Glinka *et al.*, *Nature* 391:357-62 (1998)). It was recognized as a factor capable of inducing ectopic head formation in the presence of inhibition of the BMP pathway. It was then also found to inhibit the axis-inducing activity of several *Xenopus* Wnt molecules by acting as an extracellular antagonist of Wnt signaling. Mammalian homologs have been found including Dkk-1, Dkk-2, Dkk-3, Dkk-4 and soggy (Fedi *et al.*, 1999 and Krupnick *et al.* 1999). Human Dkk-1 was also referred to as sk (Fedi *et al.* 1999). As used herein, Dkk-1 is

meant to include proteins from any species having a Wnt pathway in which Dkk-1 interacts. Particularly preferred are mammalian species (e.g., murine, caprine, canine, bovine, feline, equine, primate, ovine, porcine and the like), with particularly preferred mammals being humans. Nucleic acid sequences encoding Dkk-1 include, but are not limited to human Dkk-1 (GenBank Accession Nos. AH009834, XM_005730, AF261158, AF261157, AF177394, AF127563 and NM_012242), *Mus musculus* dickkopf homolog 1 (GenBank Accession No. NM_010051), and *Danio rerio* dickkopf-1 (GenBank Accession Nos. AF116852 and AB023488). The genomic sequences with exon annotation are GenBank Accession Nos. AF261157 and AF261158. Also contemplated are homologs of these sequences which have Dkk-1 activity in the Wnt pathway. Dkk-1 amino acid sequences include, but are not limited to human dickkopf homolog 1 (GenBank Accession Nos. AAG15544, BAA34651, NP_036374, AAF02674, AAD21087, and XP_005730), *Danio rerio* (zebrafish) dickkopf1 (GenBank Accession Nos. BAA82135 and AAD22461) and murine dickkopf-1 (GenBank Accession Nos. O54908 and NP_034181). Variants and homologs of these sequences which possess Dkk-1 activity are also included when referring to Dkk-1.

By "Dkk mediated" disorder, condition or disease is any abnormal state that involves Dkk activity. The abnormal state can be induced by environmental exposure or drug administration. Alternatively, the disease or disorder can be due to a genetic defect. Dkk mediated diseases, disorders and conditions include but are not limited to bone mass disorders or conditions and lipid disorders and conditions. For example, bone mass disorders/conditions/diseases, which may be mediated by Dkk, include but are not limited to age related loss of bone, bone fractures (e.g., hip fracture, Colle's fracture, vertebral crush fractures), chondrodystrophies, drug-induced disorders (e.g., osteoporosis due to administration of glucocorticoids or heparin and osteomalacia due to administration of aluminum hydroxide, anticonvulsants, or glutethimide), high bone turnover, hypercalcemia, hyperostosis, osteogenesis imperfecta, osteomalacia, osteomyelitis, osteoporosis, Paget's disease, osteoarthritis, and rickets.

Lipid disorders/diseases/conditions, which may be mediated by Dkk, include but are not limited to familial lipoprotein lipase deficiency, familial apoprotein CII deficiency, familial type 3 hyperlipoproteinemia, familial hypercholesterolemia, familial hypertriglyceridemia, multiple lipoprotein-type hyperlipidemia, elevated lipid levels due to dialysis and/or diabetes, and elevated lipid levels of unknown etiologies

The term "recognizes and binds," when used to define interactions of antisense nucleotides, siRNAs (small inhibitory RNA), or shRNA (short hairpin RNA) with a target sequence, means that a particular antisense, siRNA, or shRNA sequence is substantially complementary to the target sequence, and thus will specifically bind to a portion of an mRNA encoding polypeptide. As such, typically the sequences will be highly complementary to the mRNA target sequence, and will have no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 base mismatches throughout the sequence. In many instances, it may be desirable for the sequences to be exact matches, i.e. be completely complementary to the sequence to which the oligonucleotide specifically binds, and therefore have zero mismatches along the complementary stretch. As such, highly complementary sequences will typically bind quite specifically to the target sequence region of the mRNA and will therefore be highly efficient in reducing, and/or even inhibiting the translation of the target mRNA sequence into polypeptide product.

Substantially complementary oligonucleotide sequences will be greater than about 80 percent complementary (or "% exact-match") to the corresponding mRNA target sequence to which the oligonucleotide specifically binds, and will, more preferably be greater than about 85 percent complementary to the corresponding mRNA target sequence to which the oligonucleotide specifically binds. In certain aspects, as described above, it will be desirable to have even more substantially complementary oligonucleotide sequences for use in the practice of the invention, and in such instances, the oligonucleotide sequences will be greater than about 90 percent complementary to the corresponding mRNA target sequence to which the oligonucleotide specifically binds, and may in certain embodiments be greater than about 95 percent complementary to the corresponding mRNA target sequence to

which the oligonucleotide specifically binds, and even up to and including 96%, 97%, 98%, 99%, and even 100% exact match complementary to the target mRNA to which the designed oligonucleotide specifically binds.

Percent similarity or percent complementary of any of the disclosed sequences may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0, available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (1970). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess (1986), (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

By "mimetic" is meant a compound or molecule that performs the same function or behaves similarly to the compound mimicked.

By "reporter element" is meant a polynucleotide that encodes a polypeptide capable of being detected in a screening assays. Examples of polypeptides encoded by reporter elements include, but are not limited to, lacZ, GFP, luciferase, and chloramphenicol acetyltransferase.

2. Introduction

A polymorphism in LRP5 (Zmax), G171V, designated as HBM, has been identified as conferring a high bone mass phenotype in a population of related subjects as described in co-pending applications International Patent Application PCT/US 00/16951, and U.S. Patent Application Nos. 09/543,771 and 09/544,398, which are hereby incorporated by reference in their entirety (Little *et al.*, *Am J Hum Genet.* 70:11-19 (2002)). LRP5 is also described in International Patent Application WO 98/46743, which is incorporated by reference in its entirety. Loss of LRP5

function has been shown to have a deleterious effect on bone (Gong *et al.*, *Cell* 107:513-523 (2001)). Additionally, the HBM polymorphism and LRP5 may also be important in cardiac health and lipid-mediated disorders. Thus, methods of regulating their activity can serve as methods of treating and/or preventing cardiac and lipid-mediated disorders.

Recent studies have indicated that LRP5 participates in the Wnt signal transduction pathway. The Wnt pathway is critical in limb early embryological development. A recently published sketch of the components of Wnt signaling is shown in Figure 1 (Nusse, 2001 <http://www.stanford.edu/~rnusse/pathways/cell2.html>) (see also, Nusse, *Nature* 411:255-6 (2001); and Mao *et al.*, *Nature* 411:321-5 (2001)). Briefly summarized, Wnt proteins are secreted proteins which interact with the transmembrane protein Frizzled (Fz). LRP proteins, such as LRP5 and LRP6, are believed to modulate the Wnt signal in a complex with Fz (Tamai *et al.*, *Nature* 407:530-5 (2000)). The Wnt pathway acts intracellularly through the Disheveled protein (Dsh) which in turn inhibits glycogen synthetase kinase-3 (GSK3) from phosphorylating β -catenin. Phosphorylated β -catenin is rapidly degraded following ubiquitination. However, the stabilized β -catenin accumulates and translocates to the nucleus where it acts as a cofactor of the T-cell factor (TCF) transcription activator complex.

The protein dickkopf-1 (Dkk-1) is reported to be an antagonist of Wnt pathway. Dkk-1 is required for head formation in early development. Dkk-1 and its function in the Wnt pathway are described in e.g., Krupnik, *et al.*, *Gene* 238:301-13 (1999); Fedi *et al.*, *J. Biol. Chem.* 274:19465-72 (1999); see also for Dkk-1 and the Wnt pathway, Wu *et al.*, *Curr. Biol.* 10:1611-4 (2000), Shinya *et al.*, *Mech. Dev.* 98:3-17 (2000), Mukhopadhyay *et al.*, *Dev Cell* 1:423-434 (2001) and in PCT Patent Application No. WO 00/52047, and in references cited in each. It has been known that Dkk-1 acts upstream of Dsh, however the nature of the mechanism of inhibition by Dkk-1 is just beginning to be elucidated. Dkk-1 is expressed in the mouse embryonic limb bud and its disruption results in abnormal limb morphogenesis, among

other developmental defects (Gotewold *et al.*, *Mech. Dev.* 89:151-3 (1999); and, Mukhopadhyay *et al.*, *Dev Cell* 1:423-434 (2001)).

Related U.S. provisional application 60/291,311 disclosed a novel interaction between Dkk-1 (GenBank Accession No. XM 005730) and LRP5. The interaction
5 between Dkk-1 and LRP5 was discovered by a yeast two hybrid (Y2H) screen for proteins which interact with the ligand binding domain of LRP5, as described in Example 1. The two-hybrid screen is a common procedure in the art, which is described, for example, by Gietz *et al.*, *Mol. Cell. Biochem.* 172:67-79 (1997); Young, *Biol. Reprod.* 58:302-11 (1998); Brent and Finley, *Ann. Rev. Genet.* 31:663-
10 704 (1997); and Lu and Hannon, eds., Yeast Hybrid Technologies, Eaton Publishing, Natick MA, (2000). More recently, other studies confirm that Dkk-1 is a binding partner for LRP and modulates the Wnt pathway via direct binding with LRP (R. Nusse, *Nature* 411:255-256 (2001); A. Bafico *et al.*, *Nat. Cell Biol.* 3:683-686 (2001); M. Semenov, *Curr. Biol.* 11:951-961 (2001); B. Mao, *Nature* 411:321-325 (2001),
15 Zorn, *Curr. Biol.* 11:R592-5 (2001)); and, L. Li *et al.*, *J. Biol Chem.* 277:5977-81 (2002)).

Mao and colleagues (2001) identified Dkk-1 as a ligand for LRP6. Mao *et al.* suggest that Dkk-1 and LRP6 interact antagonistically where Dkk proteins inhibit the Wnt coreceptor functions of LRP6. Using co-immunoprecipitation, the group verified
20 that the Dkk-1/LRP6 interaction was direct. Dkk-2 was also found to directly bind LRP6. Contrary to data contained in provisional application 60/291,311, Mao *et al.* report that no interaction was detected between any Dkk protein and LRP5, as well as no interaction with LDLR, VLDLR, ApoER, or LRP). Additionally, Mao *et al.* demonstrated that LRP6 can titrate Dkk-1's effects of inhibiting Wnt signaling using
25 the commercial TCF-luciferase reporter gene assay (TOPFLASH). A similar conclusion was drawn from analogous studies in *Xenopus* embryos. Deletion analyses of LRP6 functional domains revealed that EGF repeats (beta-propellers) 3 and 4 were necessary for Dkk-1 binding and that the ligand binding domains of LRP6 had no effect on Dkk-1 binding. The findings of Mao *et al.* contrast with data
30 obtained by the present inventors indication that the ligand binding domains of LRP5

were necessary and sufficient for Dkk-1 binding in yeast. Using classical biochemical ligand-receptor studies, Mao *et al.* determined a $K_d=0.34$ nM for Dkk-1/LRP6 and a $K_d=0.73$ nM for Dkk-2/LRP6.

5 Semenov *et al.* (2001) verified the Mao group's results and confirmed by coimmunoprecipitation that Dkk-1 does not directly bind to Wnt or Frizzled but rather interacts with LRP6. Their Scatchard analyses found a $K_d=0.5$ nM for Dkk-1/LRP6. Semenov *et al.* also demonstrated that Dkk-1 could abolish an LRP5/Frizzled8 complex implying that Dkk-1 can also repress Wnt signaling via interactions with LRP5. A Dkk-1 mutant where cysteine 220 was changed to alanine abolished LRP6
10 binding and was unable to repress Wnt signaling. Studies in *Xenopus* embryos confirmed the results and revealed a functional consequence of Dkk-1/LRP6: repression of Wnt signaling. Their *Xenopus* work also suggested that LRP6/Dkk-1 may be specific for the canonical, β -catenin-mediated, Wnt pathways as opposed to the Wnt Planar Cell Polarity pathway.

15 Bafico *et al.* (2001) employed a 125 I-labeled Dkk-1 molecule to identify LRP6 as its sole membrane receptor with a $K_d=0.39$ nM. Again, the functional consequences of the Dkk-1/LRP6 interaction was a repression of the canonical Wnt signaling even when Dkk-1 was added at extremely low concentrations (30 pM).

20 Not wishing to be bound by theory, it is believed that the present invention provides an explanation for the mechanism of Dkk-1 inhibition of the Wnt pathway and provides a mechanism whereby the Wnt pathway may be modulated. The present application and related provisional application 60/291,311 describe Dkk-1/LRP5 interactions and demonstrate that the interaction between LRP5/LRP6/HBM and Dkk can be used in a method as an intervention point in the Wnt pathway for an
25 anabolic bone therapeutic or a modulator of lipid metabolism.

As detailed below, in the section "Methods to Identify Binding Partners" and Examples 6 and 7, Dkk-1 is able to repress LRP5-mediated Wnt signaling but not HBM-mediated Wnt signaling. This observation is of particular interest because the HBM mutation in LRP5 is a gain of function or activation mutation. That is, Wnt
30 signaling, via the canonical pathway, is enhanced with HBM versus LRP5. The

present data suggest the mechanism of this functional activation: the inability of Dkk-1 to repress HBM-mediated Wnt signaling. Further investigations of other Wnt or Dkk family members show differential activities in the canonical Wnt pathway that demonstrate the complexity and variability in Wnt signaling that can be achieved depending on the LRP/Dkk/Wnt/Frizzled repertoire that is expressed in a particular cell or tissue. This may attest to the apparent bone specificity of the HBM phenotype in humans and in the HBM transgenic animals.

Furthermore, the present data reveal the importance and functional consequence for the potential structural perturbation of the first beta-propeller domain of LRP5. Our data identified the ligand binding domain of LRP5 as the interacting region with Dkk-1 while the Mao *et al.* publication demonstrated the functional role of propellers 3 and 4 in their LRP6/Dkk-1 studies. In the present invention, we implicate the first beta propeller domain, via the HBM mutation at residue 171, as having a functional consequence in the Dkk-1-mediated Wnt pathway. The involvement of position 171 of propeller 1 may be direct or indirect with Dkk-1. Direct involvement could arise from perturbations of the 3-dimensional structure of the HBM extracellular domain that render Dkk-1 unable to bind. Alternatively, residue 171 of propeller 1 may directly interact with Dkk-1; however, by itself, it is insufficient to bind and requires other LRP5 domains. Potential indirect candidate molecules may be among the proteins identified the Dkk-1 yeast-two-hybrid experiments.

It may be that the disruption of Dkk activity is not necessarily mediated by enhancing or preventing the binding of Dkk to LRP5/LRP6/HBM. More than one mechanism may be involved. Indeed, the inventors have observed that Dkk-1 binds LRP5, LRP6, and HBM. It is able to effectively inhibit LRP6, and to a slightly lesser extent, LRP5 activity. Further, has been observed that different members of the Dkk family differentially affect LRP5/LRP6/HBM activity. For example, Dkk-1 inhibits LRP5/LRP6/HBM activity while another Dkk may enhance LRP5/LRP6/HBM activity. An endpoint to consider is the modulation of the LRP5/LRP6/HBM activity, not simply binding.

The present disclosure shows that targeting the disruption of the Dkk-1/LRP5 interaction is a therapeutic intervention point for an HBM mimetic agent. A therapeutic agent of the invention may be a small molecule, peptide or nucleic acid aptamer, antibody, or other peptide/protein, etc. Methods of reducing Dkk-1 expression may also be therapeutic using methodologies such as: RNA interference, antisense oligonucleotides, morpholino oligonucleotides, PNAs, antibodies to Dkk-1 or Dkk-1 interacting proteins, decoy or scavenger LRP5 or LRP6 receptors, and knockdown of Dkk-1 or Dkk-1 interactor transcription.

In an embodiment of the present invention, the activity of Dkk-1 or the activity of a Dkk-1 interacting protein may be modulated for example by binding with a peptide aptamer of the present invention. In another embodiment, LRP5 activity may be modulated by a reagent provided by the present invention (e.g., a peptide aptamer). In another embodiment, the Dkk-1/LRP5 interaction may be modulated by a reagent of the present invention (e.g., a Dkk-1 interacting protein such as those identified in Figure 5). In another embodiment, the Wnt signal transduction pathway may be modulated by use of one or more of the above methods. In a preferred embodiment of the present invention, the Dkk-1 mediated activity of the Wnt pathway may be specifically modulated by one or more of the above methods. In another preferred embodiment of the present invention, the Wnt signal transduction pathway may be stimulated by down-regulating Dkk-1 interacting protein activity; such down-regulation could, for example, yield greater LRP5 activity. In a more preferred embodiment, by stimulating LRP5 activity, bone mass regulation may be stimulated to restore or maintain a more optimal level. In another preferred embodiment, by stimulating LRP5 activity, lipid metabolism may be stimulated to restore or maintain a more optimal level. Alternative embodiments provide methods for screening candidate drugs and therapies directed to correction of bone mass disorders or lipid metabolism disorders. And, preferred embodiments of the present invention provide drugs and therapies developed by the use of the reagents and/or methods of the present invention. One skilled in the art will understand that the present invention provides important research tools to develop an effective model of

osteoporosis, to increase understanding of bone mass and lipid modulation, and to modulate bone mass and lipid metabolism.

Previous investigation of a large family in which high bone mass is inherited as a single gene (autosomal dominant) trait (HBM-1) has provided important insight into the mechanism by which bone density might be modulated. Members of this family have significantly increased spinal and hip BMD (>3 standard deviations above the norm) which affects young adults as well as elderly family members into the ninth decade. The bones of affected members, while appearing very dense radiographically, have normal external shape and outer dimensions. Cortical bone is thickened on endosteal surfaces and "affected" individuals are asymptomatic without any other phenotypic abnormalities. Assays of biochemical markers that reflect skeletal turnover suggest that the disorder is associated with a normal rate of bone remodeling. Affected individuals have achieved a balance in bone turnover at a density that is significantly greater than necessary for normal skeletal stresses. Importantly, the bones most affected are load-bearing bones which are subjected to the greatest mechanical and gravitational stresses (spine and hip). These are the most important bones to target for therapeutic interventions in osteoporosis. The gene identified as being responsible for this phenotype, Zmax or LRP5, was not previously associated with bone physiology. The fact that modification of this gene, such as that produced by the polymorphism leading to the autosomal dominant inheritance of the HBM family phenotype, identifies Zmax/LRP5 and the pathway by which it is regulated, including Dkk/Wnt pathways discussed above, as an important target for developing modulators of bone density. Modulation of Zmax/LRP5 to mimic the gain in function provided by the HBM polymorphism would be expected to provide an important therapy for bone wasting conditions. Additionally, such modulation in young adults could enhance peak bone mass and prevent or delay fracture risk later in life. Alternatively, modulation to reduce function could be employed to treat conditions where bone is being inappropriately produced.

3. Polypeptides

Polypeptides contemplated for use in this invention include those which modulate Dkk and Dkk interacting protein activities. Preferred polypeptides and peptides include those which modulate the Wnt pathway. Examples of preferred sequences include the Y2H baits exemplified in Figure 2, peptide aptamers of Figure 3 (SEQ ID NOs:171-188) and Figure 4 (SEQ ID NOs:189-192), the polypeptides of the Dkk-1 interacting proteins identified in Figure 5, those polypeptides shown in Figure 6, the LRP binding domain of Dkk (amino acids 138-266 of hDkk1), the cysteine-rich domain 2 (a.a. 183-245 of hDkk-1), the cysteine-rich domain 1 (a.a. 97-138 of hDkk), and LRP5 binding aptamers of Figure 13 (including SEQ ID NOs:204-213). Although Dkk-1 is exemplified, the other Dkk proteins contain substantially similar regions and may also be used according to the present invention.

For example, the baits depicted in Figure 2 were used in a yeast two hybrid (Y2H) screen. The Y2H screen was performed as described in Example 2 to determine the minimum required binding domain for Dkk-1 to bind LRP5. The minimum binding domain constructs (*i.e.*, residues 139-266 in bold below and residues 97-245 which are underlined, of Dkk-1) include the second cysteine rich domain which has sequence homology to a colipase fold.

mmalgaagat rvfvmvaaa lgghp1lgvs atlnsvlnsn aiknlppplg gaaghpgsav 60
*saapgilypg gnkyqtidny qpypcaedee cgtdeycasp **trggdagvqi clacrkrkr** 120*
cmrhamccpg nyckngicys sdqnhfrgei eetitesfgn dhstldgyar rttlaskmyh 180
tkgqegsvcl rssdcasglc carhfwskic kpvlkeggvc tkhrrkgshq leifqrcycq 240
eglscrigkd hhqasnssrl htcgrh (GenBank Accession No. XP_005730) (SEQ ID
 NO:128).

This homology suggests a lipid-binding function and may facilitate Dkk-1 interactions at the plasma membrane (van Tilbeurgh, H., *Biochim. Biophys. Acta.* 1441:173-84 (1999)). An interaction domain of Dkk-1 that is able to interact with the ligand binding domain (LBD) of LRP5 is a useful reagent in the modulation of LRP5 activity

and modulation of Dkk-1/LRP5 complex formation. Similar screens can be prepared for Dkk-1 and Dkk-1 interacting proteins or polypeptides.

A set of peptide aptamers was identified from a library of random peptides constrained and presented in a thioredoxin A (trxA) scaffold as described in Example

5 3. Peptide aptamers are powerful new tools for molecular medicine as reviewed by Hoppe-Seyler & Butz, *J. Mol. Med.*, 78:426-430 (2000); Brody and Gold, *Rev. Mol. Biotech.*, 74:5-13 (2000); and Colas, *Curr. Opin. in Chem. Biol.* 4:54-9 (2000) and the references cited therein. Briefly, peptide aptamers have been shown to be highly specific reagents capable of binding *in vivo*. As such, peptide aptamers provide a method of modulating the function of a protein and may serve as a substitute for conventional knock-out methods, knock-down or complete loss of function. Peptide aptamers are also useful reagents for the validation of targets for drug development and may be used as therapeutic compounds directly or provide the necessary foundation for drug design. Once identified, the peptide insert may be synthesized and used directly or incorporated into another carrier molecule. References reviewed and cited by Brody and Gold (2000, *supra*) describe demonstrated therapeutic and diagnostic applications of peptide aptamers and would be known to the skilled artisan.

20 The peptide aptamers of the present invention are useful reagents in the binding of Dkk-1 to its ligands and thereby modulation of the Wnt pathway and may be used to prevent Dkk-1 from inhibiting LRP5 modulation or Dkk-1 interacting protein modulation of the Wnt pathway. The sequence of these peptide aptamers is shown in Figure 3 (SEQ ID NOs:171-188). The peptide aptamers refers to the peptide constrained by the thioredoxin scaffold. The aptamers are also contemplated as therapeutic agents to treat Dkk-1 mediated diseases and conditions. Such aptamers are useful structural guides to chemists, for the design of mimetic compounds of the aptamers.

25 Peptide aptamers were likewise developed to the LRP5 ligand binding domain (LBD) bait sequences. The sequences of these peptide aptamers is shown in Figure 4 (SEQ ID NOs:189-192). These are useful reagents which may be used to disrupt

the Dkk-1/LRP5 binding interface while leaving Dkk-1 undisturbed. These can be used as comparative controls for Wnt signaling, thus, a control is provided for the specificity of any drug or therapy screened. The aptamers are also useful therapeutic agents to treat LRP mediated diseases and conditions. Such aptamers may also be used as structural guides to chemists, for the design of mimetic compounds of the aptamers.

Thirty proteins were identified which interact with Dkk-1, Dkk-1 interacting proteins, were identified in a yeast-two-hybrid screen using the Dkk-1 bait and are shown in Figure 5. It was noted that these results suggest an interaction of Dkk-1 with Notch-2. It has been suggested that cross-talk exists between the Wnt and Notch signaling pathways. For instance, Presenilin1 (Ps1) is required for Notch processing and inhibits the downstream Wnt pathway. The extracellular domain of Notch is thought to interact with Wnt. Furthermore, the Notch intracellular domain is thought to interact with disheveled and in signal induced processing, the intracellular domain is thought to interact with presenilin. (Soriano *et al.*, *J. Cell Biol.* 152:785-94 (2001)). For additional information regarding the relationships between Notch and Wnt signaling, see Wesley, *Mol. Cell. Biol.* 19:5743-58 (1999) and Axelrod *et al.*, *Science* 271:1826-32 (1996).

An interaction between Dkk-1 and chordin has also been noted; suggesting that cross-talk exists between the Wnt and TGF-beta/BMP signaling pathways (Letamendia *et al.*, *J. Bone Joint Surg. Am.* 83A:S31 (2001); Labbe *et al.*, *Proc. Natl. Acad. Sci. USA* 97:8358-63 (2000); Nishita *et al.*, *Nature* 403:781-5 (2000); DeRobertis *et al.*, *Int. J. Dev. Biol.* 45:1389-97 (2001); and Saint-Jeannet *et al.*, *Proc. Natl. Acad. Sci. USA* 94:13713-8 (1997)). The BMP signaling pathway has an established role in bone and connective tissue development, repair and homeostasis (review in Rosen and Wozney "Bone Morphogenetic Proteins" In: *Principles of Bone Biology*, 2nd Edition, Eds. J. Bilezikian, L. Raisz and G. Rodan, Academic Press, pp. 919-28 (2002)). Chordin is an important molecule during development which also modulates BMP signaling in adults by sequestering BMPs in latent complexes (Piccolo *et al.*, *Cell* 86:589-98 (1996) reviewed in Reddi, *Arthritis Res.* 3:1-5 (2001);

DeRobertis *et al.*, *Int. J. Dev. Biol.* 45:189-97 (2001)). It may be that Dkk effects bone mass modulation through both the Wnt signaling pathway via LRP and the BMP pathway via chordin.

Moreover, a number of putative growth factors, growth factor related proteins, and extracellular matrix proteins have been identified as Dkk-1 interacting proteins. Additional information regarding Dkk-1 interacting proteins identified in the Y2H assay may be obtained from publicly available databases such as PubMed via the use of the accession numbers provided in the present application. In a preferred embodiment of the invention, the amino acid sequences of these Dkk-1 interacting proteins or biologically active fragments thereof be used to modulate Dkk, Dkk-1, LRP5, LRP6, HBM, or Wnt activity. Although these proteins were identified as interacting with Dkk-1, due to the substantial homology between the various Dkk proteins, such interacting proteins are contemplated to interact with the other Dkk family members.

4. Aptamer Mimetics

The present invention further provides for mimetics of Dkk, particularly Dkk-1, and LRP5 peptide aptamers. Such aptamers may serve as structural guides to chemists for the design of mimetic compounds of the aptamers. The aptamers and their mimetics are useful as therapeutic agents to treat LRP- or Dkk-mediated diseases and conditions.

5. Nucleic Acid Molecules

The present invention further provides nucleic acid molecules that encode polypeptides and proteins which interact with Dkk and Dkk interacting proteins, and/or LRP5 (also LRP6 and HBM) to modulate biological activities of these proteins. Preferred embodiments provide nucleic acids encoding for fragments of Dkk-1 protein, including the nucleic acids of Figure 7, the Dkk-1 interacting proteins listed in Figure 5, polypeptide aptamers of Dkk-1 (Figure 3 - SEQ ID NOs:171-188), LRP5 (Figure 4 - SEQ ID NOs:189-192), Figure 13 peptide aptamers (including SEQ

ID NO:204-214) encoded by Figure 12 polynucleotides (including SEQ ID NO:193-203), LRP6 and HBM and the related fusion proteins herein described, preferably in isolated or purified form. As used herein, "nucleic acid" is defined as RNA, DNA, or cDNA that encodes a peptide as defined above, or is complementary to a nucleic acid sequence encoding such peptides, or hybridizes to either the sense or antisense strands of the nucleic acid and remains stably bound to it under appropriate stringency conditions. The nucleic acid may encode a polypeptide sharing at least about 75% sequence identity, preferably at least about 80%, and more preferably at least about 85%, with the peptide sequences; at least about 90%, 95%, 96%, 97%, 98%, and 99% or greater are also contemplated. Specifically contemplated are genomic DNA, cDNA, mRNA, antisense molecules, enzymatically active nucleic acids (e.g., ribozymes), as well as nucleic acids based on an alternative backbone or including alternative bases, whether derived from natural sources or synthesized. Such hybridizing or complementary nucleic acids, however, are defined further as being novel and nonobvious over any prior art nucleic acid including that which encodes, hybridizes under appropriate stringency conditions, or is complementary to a nucleic acid encoding a protein according to the present invention.

As used herein, the terms "hybridization" (hybridizing) and "specificity" (specific for) in the context of nucleotide sequences are used interchangeably. The ability of two nucleotide sequences to hybridize to each other is based upon the degree of complementarity of the two nucleotide sequences, which in turn is based on the fraction of matched complementary nucleotide pairs. The more nucleotides in a given sequence that are complementary to another sequence, the greater the degree of hybridization of one to the other. The degree of hybridization also depends on the conditions of stringency which include temperature, solvent ratios, salt concentrations, and the like. In particular, "selective hybridization" pertains to conditions in which the degree of hybridization of a polynucleotide of the invention to its target would require complete or nearly complete complementarity. The complementarity must be sufficiently high so as to assure that the polynucleotide of

the invention will bind specifically to the target nucleotide sequence relative to the binding of other nucleic acids present in the hybridization medium. With selective hybridization, complementarity will be about 90-100%, preferably about 95-100%, more preferably about 100%.

5 "Stringent conditions" are those that (1) employ low ionic strength and high temperature for washing, for example: 0.015 M NaCl, 0.0015 M sodium titrate, 0.1% SDS at 50°C; or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer at pH 6.5
10 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5X SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5X Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2X SSC and 0.1% SDS. A skilled artisan can readily determine
15 and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal.

As used herein, a nucleic acid molecule is said to be "isolated" or "purified" when the nucleic acid molecule is substantially separated from contaminant nucleic acid encoding other polypeptides from the source of nucleic acid. Isolated or purified
20 is also meant to include nucleic acids which encode Dkk or fragments thereof which lack surrounding genomic sequences that flank the *Dkk* gene. Isolated or purified is further intended to include nucleic acids which encode Dkk interacting proteins or biologically active fragments thereof which lack surrounding genomic sequences that flank the Dkk interacting protein genes.

25 The present invention further provides fragments of the encoding nucleic acid molecule. As used herein, a fragment of an encoding nucleic acid molecule refers to a small portion of the entire protein encoding sequence. The size of the fragment will be determined by the intended use. For example, if the fragment is chosen so as to encode an active portion of the protein, the fragment will need to be large enough
30 to encode the functional region(s) of the protein. If the fragment is to be used as a

nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming.

Fragments of the encoding nucleic acid molecules of the present invention (*i.e.*, synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding proteins of the invention can easily be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci *et al.* (*J. Am. Chem. Soc.* 103:3185-3191 (1981)) or using automated synthesis methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the gene, followed by ligation of oligonucleotides to build the complete modified gene.

The polypeptide encoding nucleic acid molecules of the present invention may further be modified to contain a detectable label for diagnostic and probe purposes. A variety of such labels are known in the art and can readily be employed with the encoding molecules herein described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides and the like. A skilled artisan can employ any of the art known labels to obtain a labeled encoding nucleic acid molecule.

Modifications to the primary structure itself by deletion, addition, or alteration of the amino acids incorporated into the protein sequence during translation can be made without destroying the activity of the protein. Such substitutions or other alterations result in proteins having an amino acid sequence encoded by a nucleic acid falling within the contemplated scope of the present invention.

Antisense molecules corresponding to the polypeptide coding or complementary sequence may be prepared. Methods of making antisense molecules which bind to mRNA, form triple helices or are enzymatically active and cleave TSG RNA and single stranded DNA (ssDNA) are known in the art. See, *e.g.*, Antisense and Ribozyme Methodology: Laboratory Companion (Ian Gibson, ed., Chapman & Hall, 1997) and Ribozyme Protocols: Methods in Molecular Biology (Phillip C. Turner, ed., Humana Press, Clifton, NJ, 1997).

Also contemplated is the use of compounds which mediate postranscriptional gene silencing (PTGS), quelling and RNA interference (RNAi). These compounds typically are about 21 to about 25 nucleotides and are also known as short interfering RNAs or short inhibitory RNAs (siRNAs). The siRNAs are produced from an initiating double stranded RNA (dsRNA). Although the full mechanism by which the siRNAs function is not fully elucidated, it is known that these siRNAs transform the target mRNA into dsRNA, which is then degraded. Preferred forms are 5' phosphorylated siRNAs, however, hydroxylated forms may also be utilized. For additional background regarding the preparation and mechanism of siRNAs generally, see, e.g., Lipardi *et al.*, *Cell* 107(3): 297-307 (2001); Boutla *et al.*, *Curr. Biol.* 11(22): 1776-80 (2001); Djikeng *et al.*, *RNA* 7(11): 1522-30 (2001); Elbashir *et al.*, *EMBO J.* 20(23): 6877-88 (2001); Harborth *et al.*, *J. Cell. Sci.* 114(Pt. 24): 4557-65 (2001); Hutvagner *et al.*, *Science* 293(5531): 811-3 (2001); and Elbashir *et al.*, *Nature* 411:494-98 (2001).

Also contemplated are short hairpin RNAs (shRNAs). shRNAs are a modification of the siRNA method described above. Instead of transfecting exogenously synthesized dsRNA into a cell, sequence-specific silencing can be achieved by stably expressing siRNA from a DNA template as a fold-back stem-loop, or hairpin. This approach is known as shRNA. This method permits the analysis of loss of function phenotypes due to sequence-specific gene silencing in mammalian cells by avoiding many of the problems associated with siRNAs, such as RNase degradation of the reagents, expensive chemical synthesis, etc. For additional background regarding the preparation and mechanism of shRNAs generally, see, e.g., Yu *et al.*, *PNAS* 99:6047-6052 (2002); Paddison *et al.*, *Genes and Devel.* 16:948-58 (2002); and Brummelkamp *et al.*, *Science* 296:550-553 (2002). For additional background on the use of this method in mammalian gene knockdown methodologies, see Tuschl, *Nature Biotech.* 20:446-448 (2002) (and references therein).

In one preferred embodiment, the siRNA or shRNA is directed to a Dkk encoding mRNA, wherein a preferred Dkk is Dkk-1. In another embodiment, the

siRNA or shRNA is directed towards a protein which binds to and modulates the activity of or is modulated by a Dkk; these proteins include LRP5, LRP6 and HBM as well as other members of the Wnt pathway.

5 6. **Isolation of Other Related Nucleic Acid Molecules**

The identification of the nucleic acid molecule of Dkk allows a skilled artisan to isolate nucleic acid molecules that encode other members of the Dkk family (see, Krupnik *et al.*, 1999). Further, the presently disclosed nucleic acid molecules allow a skilled artisan to isolate nucleic acid molecules that encode Dkk-1-like proteins, in addition to Dkk-1. The presently disclosed Dkk-1 interacting proteins and their corresponding nucleic acid molecules allows a skilled artisan to further isolate other related protein family members which interact with Dkk-1.

10 A skilled artisan can readily use the amino acid sequence of Dkk and Dkk interacting proteins to generate antibody probes to screen expression libraries prepared from appropriate cells. Typically, polyclonal antiserum from mammals such as rabbits immunized with the purified protein (as described below) or monoclonal antibodies can be used to probe a mammalian cDNA or genomic expression library, such as a human macrophage library, to obtain the appropriate coding sequence for other members of the protein family. The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own control sequences, or expressed by constructions using control sequences appropriate to the particular host used for expression of the desired protein.

15 Alternatively, a portion of the coding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the protein family from any mammalian organism. Oligomers containing approximately 18-20 nucleotides (encoding about a 6-7 amino acid stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient stringency to eliminate an undue level of false positives.

20 Additionally, pairs of oligonucleotide primers can be prepared for use in a polymerase chain reaction (PCR) to selectively clone an encoding nucleic acid

molecule. A PCR denature/anneal/extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other encoding nucleic acid molecules. For example, degenerate primers can be utilized to obtain sequences related to Dkk-1 or Dkk-1 interacting proteins. Primers can be designed that are not perfectly complementary and can still hybridize to a portion of a target sequence or flanking sequence and thereby provide for amplification of all or a portion of a target sequence. Primers of about 20 nucleotides or less, preferably have about one to three mismatches located at the 5' and/or 3' ends. Primers of about 20 to 30 nucleotides have up to about 30% mismatches and can still hybridize to a target sequence.

Hybridization conditions for primers with mismatch can be determined by the method described in Maniatis *et al.*, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982) or by reference to known methods. The ability of the primer to hybridize to a sequence of either Dkk-1, a Dkk-1 interacting protein, or a related sequence under varying conditions can be determined using this method. Because a target sequence is known, the effect of mismatches can be determined by methods known to those of skill in the art. Degenerate primers would be based on putative conserved amino acid sequences of the Dkk-1 and Dkk-1 interacting protein genes.

7. rDNA Molecules for Polypeptide Expression

The present invention further provides recombinant DNA molecules (rDNAs) that contain a polypeptide coding sequence. As used herein, a rDNA molecule is a DNA molecule that has been subjected to molecular manipulation *in situ*. Methods for generating rDNA molecules are well known in the art, for example, see Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989). In the preferred rDNA molecules, a coding DNA sequence is operably linked to expression control sequences and/or vector sequences.

The choice of vector and/or expression control sequences to which one of the protein family encoding sequences of the present invention is operably linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein

expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication and/or insertion into the host chromosome, and preferably also expression, of the structural gene included in the rDNA molecule.

5 Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, and other regulatory elements. Preferably, the inducible promoter is readily controlled, such as being responsive to a nutrient in the host cell's medium. Preferred promoters include
10 yeast promoters, which include promoter regions for metallothionein, 3-phosphoglycerate kinase or other glycolytic enzymes such as enolase or glyceraldehyde-3-phosphate dehydrogenase, enzymes responsible for maltose and galactose utilization, and others. Vectors and promoters suitable for use in yeast expression are further described in EP 73,675A. Appropriate non-native mammalian
15 promoters might include the early and late promoters from SV40 (Fiers et al, *Nature*, 273:113 (1978)) or promoters derived from Moloney murine leukemia virus, mouse tumor virus, avian sarcoma viruses, adenovirus II, bovine papilloma virus or polyoma. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other
20 expression control sequences, see also Enhancers and Eukaryotic Gene Expression (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1983). Preferred bone related promoters include CMVbActin or type I collagen promoters to drive expression of the human HBM, Zmax1/LRP5 or LRP6 cDNA. Other preferred promoters for mammalian expression are from cytomegalovirus (CMV), Rous sarcoma virus (RSV), Simian virus
25 40 (SV40), and EF-1a (human elongation factor 1a-subunit).

 In one embodiment, the vector containing a coding nucleic acid molecule will include a prokaryotic replicon, *i.e.*, a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed
30 therewith. Such replicons are well known in the art. In addition, vectors with a

prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

5 Vectors that include a prokaryotic replicon can further include a prokaryotic or bacteriophage promoter capable of directing the expression (transcription and translation) of the coding gene sequences in a bacterial host cell, such as *E. coli*. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing
10 convenient restriction sites for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA), and pPL and pKK223 available from Pharmacia (Piscataway, NJ).

15 Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can also be used to form a rDNA molecule that contains a coding sequence. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of a desired DNA segment. Typical of such vectors are pSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International
20 Biotechnologies, Inc.), vector systems that include Histidine Tags and periplasmic secretion, or other vectors described in the art.

Eukaryotic cell expression vectors used to construct the rDNA molecules of the present invention may further include a selectable marker that is effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug
25 resistance marker is the gene whose expression results in neomycin resistance, *i.e.*, the neomycin phosphotransferase (*neo*) gene (Southern *et al.*, *J. Mol. Anal. Genet.* 1:327-341 (1982)). Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors introduced by co-transfection of the host cell, and selected by culturing in the appropriate drug for the selectable marker.

8. Host Cells Containing an Exogenously Supplied rDNA Nucleic Acid Molecule

The present invention further provides host cells transformed with a nucleic acid molecule that encodes a polypeptide or protein of the present invention. The host cell can be either prokaryotic or eukaryotic. Eukaryotic cells useful for expression of a protein of the invention are not limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of the gene product. Preferred eukaryotic host cells include, but are not limited to, yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human cell line but also can include invertebrates with, for example, cartilage. Preferred eukaryotic host cells include but are not limited to Chinese hamster ovary (CHO) cells (ATCC No. CCL61), NIH Swiss mouse embryo cells NIH/3T3 (ATCC No. CRL 1658), baby hamster kidney cells (BHK), HOB-03-CE6 osteoblast cells, and other like eukaryotic tissue culture cell lines.

Any prokaryotic host can be used to express a rDNA molecule encoding a protein of the invention. A preferred prokaryotic host is *E. coli*.

Transformation of appropriate cell hosts with a recombinant DNA (rDNA) molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. With regard to transformation of prokaryotic host cells, electroporation and salt treatment methods are typically employed; see, for example, Cohen *et al.*, *Proc. Natl. Acad. Sci. USA* 69: 2110 (1972); Maniatis *et al.* (1982); and Sambrook *et al.* (1989). With regard to transformation of vertebrate cells with vectors containing rDNAs, electroporation, cationic lipid or salt treatment methods are typically employed; see, for example, Graham *et al.*, *Virology* 52: 456 (1973); Wigler *et al.*, *Proc. Natl. Acad. Sci. USA* 76: 1373-76 (1979).

Successfully transformed cells, *i.e.*, cells that contain a rDNA molecule of the present invention, can be identified by well known techniques including the selection for a selectable marker. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to produce single colonies. Cells from those

colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using a method such as that described by Southern, *J. Mol. Biol.* 98: 503 (1975), or Berent *et al.*, *Biotech.* 3: 208 (1985). Alternatively, the cells can be cultured to produce the proteins encoded by the rDNA and the proteins harvested and assayed, using for example, any suitable immunological method. See, e.g., Harlow *et al.*, (1988).

Recombinant DNA can also be utilized to analyze the function of coding and non-coding sequences. Sequences that modulate the translation of the mRNA can be utilized in an affinity matrix system to purify proteins obtained from cell lysates that associate with the Dkk-1 or Dkk-1 interacting protein or expression control sequence. Synthetic oligonucleotides would be coupled to the beads and probed with the lysates, as is commonly known in the art. Associated proteins could then be separated using, for example, a two dimensional SDS-PAGE system. Proteins thus isolated could be further identified using mass spectroscopy or protein sequencing. Additional methods would be apparent to the skilled artisan.

9. Production of Recombinant Peptides and Proteins using a cDNA or Other Recombinant Nucleic Acids

The invention also relates to nucleic acid molecules which encode a Dkk protein and polypeptide fragments thereof, and proteins and polypeptides which bind to Dkk (e.g., LRP5, LRP6 and HBM, Dkk interacting proteins such as the proteins of Figure 5) and molecular analogues. The polypeptides of the present invention include the full length Dkk and polypeptide fragments thereof, Dkk binding proteins and polypeptides thereof. Preferably these proteins are mammalian proteins, and most preferably human proteins and biologically active fragments thereof. Alternative embodiments include nucleic acid molecules encoding polypeptide fragments having a consecutive amino acid sequence of at least about 3, 5, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, or 200 amino acid residues from a common polypeptide sequence; amino acid sequence variants of a common polypeptide sequence wherein an amino acid residue has been inserted N- or C-terminal to, or within, the polypeptide sequence or its fragments; and amino acid sequence variants of the common

polypeptide sequence or its fragments, which have been substituted by another conserved residue. Recombinant nucleic acid molecules which encode polypeptides include those containing predetermined mutations by, e.g., homologous recombination, site-directed or PCR mutagenesis, and recombinant Dkk proteins or polypeptide fragments of other animal species, including but not limited to vertebrates (e.g., rabbit, rat, murine, porcine, camelid, reptilian, caprine, avian, fish, bovine, ovine, equine and non-human primate species) as well as invertebrates, and alleles or other naturally occurring variants and homologs of Dkk binding proteins of the foregoing species and of human sequences. Also contemplated herein are derivatives of the commonly known Dkk, Dkk interacting proteins, or fragments thereof, wherein Dkk, Dkk interacting proteins, or their fragments have been covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid (for example a detectable moiety such as an enzyme or radioisotope) and soluble forms of Dkk. It is further contemplated that the present invention also includes nucleic acids with silent mutations which will hybridize to the endogenous sequence and which will still encode the same polypeptide.

The nucleic acid molecules encoding Dkk binding proteins, the LRP5 binding domain fragment of Dkk, or other polypeptides of the present invention are preferably those which share a common biological activity (e.g., mediate Dkk activity such as its interaction with LRP5, HBM or LRP6). The polypeptides of the present invention include those encoded by a nucleic acid molecule with silent mutations, as well as those nucleic acids encoding a biologically active protein with conservative amino acid substitutions, allelic variants, and other variants of the disclosed polypeptides which maintain at least one Dkk activity.

The amino acid compounds of the invention are polypeptides which are partially defined in terms of amino acid residues of designated classes. Polypeptide homologs would include conservative amino acid substitutions within the amino acid classes described below. Amino acid residues can be generally sub-classified into four major subclasses as follows:

Acidic: The residue has a negative charge due to loss of H^+ ion at physiological pH, and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium, at physiological pH.

5 Basic: The residue has a positive charge due to association with H^+ ion at physiological pH, and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH.

10 Neutral/non-polar: The residues are not charged at physiological pH, but the residue is repelled by aqueous solution so as to seek the inner positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium. These residues are also designated "hydrophobic."

15 Neutral/polar: The residues are not charged at physiological pH, but the residue is attracted by aqueous solution so as to seek the outer positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium.

20 It is understood, of course, that in a statistical collection of individual residue molecules some molecules will be charged, and some not, and there will be an attraction for or repulsion from an aqueous medium to a greater or lesser extent. To fit the definition of "charged", a significant percentage (at least approximately 25%) of the individual molecules are charged at physiological pH. The degree of attraction or repulsion required for classification as polar or nonpolar is arbitrary and, therefore, amino acids specifically contemplated by the invention have been classified as one or the other. Most amino acids not specifically named can be classified on the basis of known behavior.

25 Amino acid residues can be further subclassified as cyclic or noncyclic, and aromatic or non-aromatic, self-explanatory classifications with respect to the side chain substituent groups of the residues, and as small or large. The residue is considered small if it contains a total of 4 carbon atoms or less, inclusive of the carboxyl carbon. Small residues are, of course, always nonaromatic.

The gene-encoded secondary amino acid proline, although technically within the group neutral/nonpolar/large/cyclic and nonaromatic, is a special case due to its known effects on the secondary conformation of peptide chains, and is not, therefore, included in this defined group.

5 Other amino acid substitutions of those encoded in the gene can also be included in peptide compounds within the scope of the invention and can be classified within this general scheme according to their structure.

All of the compounds of the invention may be in the form of the pharmaceutically acceptable salts or esters. Salts may be, for example, Na^+ , K^+ , Ca^{+2} , Mg^{+2} and the like; the esters are generally those of alcohols of 1-6 carbons.

10 The present invention further provides methods for producing a protein of the invention using nucleic acid molecules herein described. In general terms, the production of a recombinant form of a protein typically involves the following steps.

First, a nucleic acid molecule is obtained that encodes Dkk, such as a nucleic acid molecule encoding human Dkk or any other Dkk sequence, or that encodes a Dkk binding protein, a Dkk aptamer or a biologically active fragment thereof. Particularly for Dkk binding peptides, the nucleotides encoding the peptide are incorporated into a nucleic acid in the form of an in-frame fusion, insertion into or appended to a thioredoxin coding sequence. The coding sequence (ORF) is directly suitable for expression in any host, as it is not interrupted by introns.

20 These DNAs can be transfected into host cells such as eukaryotic cells or prokaryotic cells. Eukaryotic hosts include mammalian cells and vertebrate (e.g., osteoblasts, osteosarcoma cell lines, Drosophila S2 cells, hepatocytes, tumor cell lines and other bone cells of any mammal, as well as insect cells, such as Sf9 cells using recombinant baculovirus). For example, a DNA expressing an open reading frame (ORF) under control of a type I collagen promoter, or such osteoblast promoters as osteocalcin histone, type I collagen, TGF β 1, MSX2, cfos/cJun and Cbfa1, can be used to regulate the Dkk in animal cells. Alternatively, the nucleic acid can be placed downstream from an inducible promoter, which can then be placed into vertebrate or invertebrate cells or be used in creating a transgenic animal model.

Alternatively, proteins and polypeptides of the present invention can be expressed in an heterologous system. The human cell line GM637, SV-40 transformed human fibroblasts, can be transfected, with a plasmid containing a Dkk ligand binding domain coding sequence under the control of the chicken actin promoter (Reis *et al.*, EMBO J. 11: 185-193 (1992)). Such transfected cells could be used as a source of Dkk binding domain in functional assays. Alternatively, polypeptides encoding only a portion of Dkk or any of the disclosed Dkk binding peptides Dkk aptamers or a polypeptide encoding a Dkk interacting protein can be expressed alone or in the form of a fusion protein. For example, Dkk derived peptides can be expressed in bacteria (*e.g.*, *E. coli*) as GST- or His-Tag fusion proteins. These fusion proteins are then purified and can be used to generate polyclonal antibodies or can be used to identify other Dkk ligands.

The nucleic acid coding sequence is preferably placed in operable linkage with suitable control sequences, as described above, to form an expression unit containing the protein encoding open reading frame. The expression unit is used to transform a suitable host and the transformed host is cultured under conditions that allow the production of the recombinant protein. Optionally the recombinant protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

Each of the foregoing steps can be done in a variety of ways. For example, the desired coding sequences may be obtained from genomic fragments and used directly in appropriate hosts. The construction of expression vectors that are operable in a variety of hosts is accomplished using appropriate replicons and control sequences, as set forth above. The control sequences, expression vectors, and transformation methods are dependent on the type of host cell used to express the gene and were discussed in detail earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system known in the art for use with the nucleic acid molecules of the invention to produce recombinant protein.

10. Methods to Identify Binding Partners

Another embodiment of the present invention provides methods for use in isolating and identifying binding partners of Dkk or Dkk interacting proteins. Dkk or a Dkk interacting protein or a polypeptide fragment thereof can be mixed with a potential binding partner or an extract or fraction of a cell under conditions that allow the association of potential binding partners with Dkk or with Dkk interacting proteins. After mixing, the peptides, polypeptides, proteins or other molecules that have become associated with Dkk or a Dkk interacting protein are separated from the mixture. The binding partner that bound to the polypeptide then can be purified and further analyzed. Determination of binding partners of Dkk and Dkk interacting proteins as well as agents which prevent the interaction of Dkk with one of its interacting proteins (e.g., LRP5, LRP6, HBM, or those proteins listed in Figure 5) can be performed using a variety of different competition assays as are known in the art. For example, the minimal sequence of Dkk, as described herein, can be used to identify antibodies which compete with LRP5 (or LRP6, HBM or other ligand binding partners) for binding to Dkk-1 and vice versa. The minimal Dkk sequence can be bound to the bottom of a 96-well plate (or other solid substrate), and antibodies or other potential binding agents (e.g., polypeptides, mimetics, homologs, antibody fragments and the like) can be screened in a competition assay to identify agents with binding affinities, for example, greater than the natural ligand binding partner of Dkk.

In the present invention, suitable cells are used for preparing assays, for the expression of a LRP and/or Dkk or proteins that interact therewith. The cells may be made or derived from mammals, yeast, fungi, or viruses. A suitable cell for the purposes of this invention is one that includes but is not limited to a cell that can exhibit a detectable Dkk-LRP (or HBM) interaction, and preferably, the differential interaction between Dkk-1-LRP5 and Dkk-1-HBM. For the desired assay, the cell type may vary. In several embodiments, bone cells are preferred; for example, a human osteoblast cell (e.g. hOB-03-CE6) or osteosarcoma cell (e.g. U2OS). Additional hOB cells are hOB-03-C5, hOB-02-02 and, an immortalized pre-osteocytic cell line referred to as hOB-01-C1-PS-09 cells (which are deposited with American Type Culture Collection in

Manassas, Va. with the designation PTA-785), Examples of osteosarcoma cells would include SaoS2, MG63 and HOS TE85. Immortalized refers to a substantially continuous and permanently established cell culture with substantially unlimited cell division potential. That is, the cells can be cultured substantially indefinitely, i.e., for at least about 6 months under rapid conditions of growth, preferably much longer under slower growth conditions, and can be propagated rapidly and continually using routine cell culture techniques. Alternatively stated, preferred cells can be cultured for at least about 100, 150 or 200 population doublings. These cells produce a complement of proteins characteristic of normal human osteoblastic cells and are capable of osteoblastic differentiation. They can be used in cell culture studies of osteoblastic cell sensitivity to various agents, such as hormones, cytokines, and growth factors, or in tissue therapy. Certain non bone cells such as HEK 293 cells that exhibit detectable Dkk-LRP (or HBM) interaction are also be useful for the assays of this invention.

To identify and isolate a binding partner, the entire Dkk protein (e.g., human Dkk-1, GenBank Accession No. BAA34651) or a Dkk interacting protein (Genbank Accession Nos. for some Dkk-1 interacting proteins are given in Figure 5) can be used. Alternatively, a polypeptide fragment of the protein can be used. Suitable fragments of the protein include at least about 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150 or more contiguous amino acid residues of any Dkk or Dkk interactor sequence. Preferable sequences of Dkk include portions or all of one or both of the cysteine rich domains (e.g., Cys-1 and Cys-2 of Dkk-1) or the conserved sequences at the amino terminus of Dkk-1 (See Krupnik *et al.*, *Gene* 238: 301-313 (1999)). Alternatively, portions of LRP5, LRP6, HBM and other Dkk interacting proteins such as those in Figure 5 that interact with Dkk-1 can be used to identify and isolate agents which modulate Dkk activity. Alternatively, peptide aptamers of LRP5, LRP6, HBM, Dkk and other Dkk interacting proteins such as those in Figure 5 that interact with Dkk-1 can be used to identify and isolate agents which modulate Dkk activity.

As used herein, a cellular extract refers to a preparation or fraction which is made from a lysed or disrupted cell. A variety of methods can be used to obtain cell

extracts. Cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods include, but are not limited to, detergent lysis and enzyme lysis. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain extracts for use in the present methods.

Once an extract of a cell is prepared, the extract is mixed with the protein of the invention under conditions in which association of the protein with the binding partner can occur. A variety of conditions can be used, the most preferred being conditions that closely resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH, temperature, and the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner.

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be utilized to separate the mixture. For example, antibodies specific to a protein of the invention can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density/sediment centrifugation can be used. For example, a protein of the invention is expressed with an affinity tag such as a His tag. The His labeled protein and any bound molecule may be retained and selectively eluted from a Ni-NTA column.

After removal of non-associated cellular constituents found in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

To aid in separating associated binding partner pairs from the mixed extract, the protein of the invention can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the protein to a solid support aids in separating peptide/binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of two or more proteins.

Alternatively, the nucleic acid molecules of the invention can be used in a Y2H system. The Y2H system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described. Methods of performing and using Y2H systems are known. See, e.g., Finley *et al.*, "Two-Hybrid Analysis of Genetic Regulatory Networks," in The Yeast Two-Hybrid System (Paul L. Bartel *et al.*, eds., Oxford, 1997); Meijia Yang, "Use of a Combinatorial Peptide Library in the Two-Hybrid Assay," in The Yeast Two-Hybrid System (Paul L. Bartel *et al.*, eds., Oxford, 1997); Gietz *et al.*, "Identification of proteins that interact with a protein of interest: Applications of the yeast two-hybrid system," *Mol. & Cell. Biochem.* 172: 67-9 (1997); K. H. Young, "Yeast Two-Hybrid: So Many Interactions,(in) so Little Time," *Biol. Reprod.* 58: 302-311 (1998); R. Brent *et al.*, "Understanding Gene and Allele Function with Two-Hybrid Methods," *Annu. Rev. Genet.* 31:663-704 (1997) and U.S. Patent No. 5,989,808. The Dkk-1 interacting proteins identified in Figure 5 were identified using the Y2H interacting system using Dkk-1 as bait.

One preferred *in vitro* binding assay for Dkk modulators would comprise a mixture of a LRP binding domain of Dkk and one or more candidate binding targets or substrates. After incubating the mixture under appropriate conditions, one would determine whether Dkk or a fragment thereof bound with the candidate modulator present. For cell-free binding assays, one or more of the components usually comprises or is coupled to a label. The label may provide for direct detection, such as radioactivity, luminescence, optical or electron density, *etc.*, or indirect detection such as an epitope tag, an enzyme, *etc.* A variety of methods may be employed to detect the label depending on the nature of the label and other assay components. For example, the label may be detected bound to the solid substrate or a portion of the bound complex containing the label may be separated from the solid substrate, and the label thereafter detected. Fluorescence resonance energy transfer may be utilized to monitor the interaction of two labeled molecules. For example, a fluorescence label on Dkk and another label on LRP5 or a soluble fragment thereof such as the extracellular domain will exchange fluorescence resonance energy when in close proximity indicating that the two molecules are bound. A preferred binding partner for Dkk will

increase or decrease the affinity between Dkk and LRP5 which will be readily observable in a fluorescence spectrometer. Alternatively, an instrument, such as a surface plasmon resonance detector manufactured by BIAcore (Uppsala, Sweden), may be used to observe interactions with a fixed target. One skilled in the art knows of many other methods which may be employed for this purpose.

Thereby, the present invention provides methods for screening candidates including polypeptides of the present invention for activity which identifies these candidates as valuable drug leads. Other suitable methods are also known in the art and are suitable for use herein, including *Xenopus* oocyte injection studies and TCF luciferase assays.

Additional assays can be used to identify the activity of Dkk and Dkk interacting proteins in the Wnt pathway, as well as the impact of modulators of Dkk and Dkk interacting proteins on the Wnt pathway. These include, for example, a *Xenopus* embryo assay and a TCF-luciferase reporter gene assay to monitor Wnt signaling modulation.

Xenopus embryos are an informative *in vivo* assay system to evaluate the modulation of Wnt signaling. Ectopic expression of certain Wnts or other activators of the Wnt signaling pathway results in a bifurcation of the anterior neural plate. This bifurcation results in a duplicated body axis, which suggests a role for Wnt signaling during embryonic development (McMahon *et al.*, *Cell* 58: 1075-84 (1989); Sokol *et al.*, *Cell* 67: 741-52 (1991)). Since these original observations, the *Xenopus* embryo assay has been extensively used as an assay system for evaluating modulation of the Wnt signaling pathway. One preferred embodiment of the present invention is demonstrated in Example 6.

Constructs for *Xenopus* expression can be prepared as would be known in the art. For example, a variety of cDNAs have been engineered into the vector pCS2+ (Turner *et al.*, *Genes Devel.* 8: 1434-1447 (1994)) to facilitate the *in vitro* generation of mRNA for use in *Xenopus* embryo injection experiments. DNA inserts are subcloned in the sense orientation with respect to the vector SP6 promoter. Downstream of the insert, the vector provides an SV40 virus polyadenylation signal and a T3 promoter

sequence (*i.e.*, for the generation of antisense mRNA). Constructs can be generated for various Dkk family members, LRP5, LRP6, HBM, Dkk-1 interactors, etc. Constructs could be generated in pCS2⁺ that contain the nucleic acid sequence encoding for the peptide aptamers that were identified in yeast screens. These sequences would be fused to a 5' synthetic translation initiation sequence followed by a canonical signal sequence to ensure that the peptide aptamer would be translated and secreted from the cell.

Once these constructs are made then mRNA can be synthesized and injected into *Xenopus* oocytes. mRNA for microinjection into *Xenopus* embryos is generated by *in vitro* transcription using the cDNA constructs in the pCS2⁺ vector described above as template. Various amounts of RNA can be injected into the ventral blastomere of the 4- or 8-cell *Xenopus* embryo substantially as described in Moon *et al.*, *Technique-J. of Methods in Cell and Mol. Biol.* 1: 76-89 (1989), and Peng, *Meth. Cell. Biol.* 36: 657-62 (1991).

Previous data has shown that expression of LRP5, in the presence of Wnt5a, results in a Wnt-induced duplicated axis formation in *Xenopus* embryos (Tamai *et al.*, *Nature* 407: 530-535 (2000)). The roles of Dkk-1 and Dkk-2, and Dkk-1 interacting proteins, in modulating the LRP5-mediated Wnt response *in vivo* can be analyzed using, for example, the *Xenopus* embryo. In addition, the peptide aptamers, Dkk interacting proteins, or combinations of the above can be evaluated in a similar manner.

Experiments can also be conducted wherein RNA is injected into the dorsal blastomere to ensure the specificity of the observed phenotypes. Lineage tracing experiments can be performed where a marker gene such as green fluorescent protein (GFP) or LacZ is co-injected with the experimental RNAs. Detecting marker gene expression would identify the targeted cells of the microinjection and aid in elucidating the mechanism of action. In addition to the Wnt signaling components listed above, the point at which HBM acts upon the Wnt pathway can also be analyzed. This can be done by co-injections of various dominant-negative constructs. For example, a dominant negative TCF-3 construct would be useful to demonstrate that the observed axis duplication (and Wnt activation) is mediated via the β -catenin-TCF response. If so,

such a construct would be expected to abolish the observed duplicated axis phenotype. Another example would include a dominant negative Dsh construct. Since Dsh is far upstream in the Wnt signaling pathway, a dominant negative construct should abolish the activation of the Wnt response and the observed axis duplication. If it does not, this would suggest that axis duplication is being induced via a different signaling pathway.

The marker genes of the injected *Xenopus* embryos can be analyzed as follows. Representative embryos are collected at stage 10.5 (11 hours post fertilization) for marker gene analysis. RNA is extracted and purified from the embryos following standard protocols (Sambrook *et al.*, 1989 at 7.16). Marker genes could include the following: Siamois (*i.e.*, Wnt responsive gene), Xnr3 (*i.e.*, Wnt responsive gene), slug (*i.e.*, neural crest marker), Xbra (*i.e.*, early mesoderm marker), HNK-1 (*i.e.*, ectodermal/neural marker), endodermin (*i.e.*, endoderm), Xlhx8 (*i.e.*, pancreatic), BMP2 and BMP4 (*i.e.*, early mesoderm), XLRP6 (*i.e.*, maternal and zygotic expression, it is also the LRP6 homolog in the frog), EF-1 (*i.e.*, control) and ODC (*i.e.*, control).

Induction of marker genes is analyzed and quantitated by RT-PCR/TaqMan®.

This type of marker analysis is excellent to monitor changes in gene expression that result very early in the embryo as a direct result of signaling perturbation. Other experiments could be designed that would monitor changes in gene expression in a more tissue or spatially-restricted fashion. Examples would include the generation of a transgenic *Xenopus* model. For example, Zmax/LRP5 and HBM expression could be under the control of the brachyury or cardiac-actin promoters directing gene expression transiently in the mesoderm or in the somites, respectively. Phenotype analyses of these transgenic *Xenopus* animals would include marker gene analysis/transcriptional profiling (from a restricted tissue source) and histologic examination of the tissue.

A TCF-luciferase assay system such as that described in Example 7 can also be used to monitor Wnt signaling activity, Dkk activity and Dkk interacting protein activity. Constructs for the TCF-luciferase assays can be prepared as would be known in the art. For example, Dkk and Dkk interacting protein peptides, LRP5/LRP6, among others, can be expressed in pcDNA3.1, using Kozak and signal sequences to target peptides for secretion.

Once constructs have been prepared, cells such as osteoblasts and HEK293 cells are seeded in well plates and transfected with construct DNA, CMV beta-galactosidase plasmid DNA, and TCF-luciferase reporter DNA. The cells are then lysed and assayed for beta-galactosidase and luciferase activity to determine whether Dkk, Dkk interacting proteins, or other molecules such as antibodies affect Wnt signaling.

Additional assays for monitoring Wnt signaling activity, Dkk activity, and Dkk interacting protein activity include:

Modulation of another Wnt-responsive transcription factor, LEF, as visualized by a reporter gene activity. One example includes the activation of the LEF1 promoter region fused to the luciferase reporter gene (Hsu *et al.*, *Mol. Cell. Biol.* 18: 4807-18 (1999)).

Alterations in cell proliferation, cell cycle or apoptosis. There are numerous examples describing Wnt-mediated cellular transformations including Shimizu *et al.*, *Cell. Growth Differ.* 8: 1349-58 (1997).

Stabilization and cellular localization of de-phosphorylated β -catenin as an indicator of Wnt activation (Shimizu *et al.*, 1997).

Additional methods of assaying Wnt signaling, through either the canonical or non-canonical pathways, would be apparent to the artisan of ordinary skill.

11. Methods to Identify Agents that Modulate the Expression of a Nucleic Acid Encoding the Dkk and/or LRP5 Proteins and/or Dkk interacting proteins

Another embodiment of the present invention provides methods for identifying agents that modulate the expression of a nucleic acid encoding Dkk. Such assays may utilize any available means of monitoring for changes in the expression level of the nucleic acids of the invention. As used herein, an agent is said to modulate the expression of Dkk, if it is capable of up- or down-regulating expression of the nucleic acid in a cell (e.g., mRNA).

In one assay format, cell lines that contain reporter gene fusions between the nucleic acid encoding Dkk (or proteins which modulate the activity of Dkk) and any

assayable fusion partner may be prepared. Numerous assayable fusion partners are known and readily available, including but not limited to the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.*, *Anal. Biochem.* 188: 245-254 (1990)). Cell lines containing the reporter gene fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid encoding Dkk or other protein which modulates Dkk activity. Such assays can similarly be used to determine whether LRP5 and even LRP6 activity is modulated by regulating Dkk activity.

Additional assay formats may be used to monitor the ability of the agent(s) to modulate the expression of a nucleic acid encoding Dkk, alone or Dkk and LRP5, and/or Dkk interacting proteins such as those identified in Figure 5. For instance, mRNA expression may be monitored directly by hybridization to the nucleic acids of the invention. Cell lines are exposed to the agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook *et al.* (1989); Ausubel *et al.*, Current Protocols in Molecular Biology (Greene Publishing Co., NY, 1995); Maniatis *et al.*, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982); and Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology (Frederick M. Ausubel *et al.*, April 1999).

Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells may be prepared from the nucleic acids of the invention. It is preferable, but not necessary, to design probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid hybrids form under conditions of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between two nucleic acid strands in order to form a hybrid. Stringency should be chosen to maximize the difference in stability between the probe:target hybrid and potential probe:non-target hybrids.

Probes may be designed from the nucleic acids of the invention through methods known in the art. For instance, the G+C content of the probe and the probe length can affect probe binding to its target sequence. Methods to optimize probe specificity are commonly available. See for example, Sambrook *et al.* (1989) or Ausubel *et al.* (Current Protocols in Molecular Biology, Greene Publishing Co., NY, 1995).

Hybridization conditions are modified using known methods, such as those described by Sambrook *et al.* (1989) and Ausubel *et al.* (1995), as suitable for each probe. Hybridization of total cellular RNA or RNA enriched for polyA RNA can be accomplished in any available format. For instance, total cellular RNA or RNA enriched for polyA RNA can be affixed to a solid support and the solid support exposed to at least one probe comprising at least one, or part of one of the nucleic acid sequences of the invention under conditions in which the probe will specifically hybridize.

Alternatively, nucleic acid fragments comprising at least one, or part of one of the sequences of the invention can be affixed to a solid support, such as a porous glass wafer. The glass or silica wafer can then be exposed to total cellular RNA or polyA RNA from a sample under conditions in which the affixed sequences will specifically hybridize. Such glass wafers and hybridization methods are widely available, for example, those disclosed by Beattie (WO 95/11755). By examining for the ability of a given probe to specifically hybridize to an RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up- or down-regulate the expression of a nucleic acid encoding Dkk, a Dkk interacting protein, and/or LRP5 can be identified.

Microarray technology and transcriptional profiling are examples of methods which can be used to analyze the impact of putative Dkk or Dkk interacting protein modulating compounds. For transcriptional profiling, mRNA from cells exposed *in vivo* to a potential Dkk modulating agent, such as the Dkk interacting proteins identified in the present invention (e.g., those identified in Figure 5), agents which modulate Dkk interacting proteins, and mRNA from the same type of cells that were not exposed to the agent could be reverse transcribed and hybridized to a chip containing DNA from

numerous genes, to thereby compare the expression of genes in cells treated and not treated with the agent. If, for example a putative Dkk modulating agent down-regulates the expression of Dkk in the cells, then use of the agent may be undesirable in certain patient populations. For additional methods of transcriptional profiling and the use of microarrays, refer to, for example, U.S. Patent No. 6,124,120 issued to Lizardi (2000).

Additional methods for screening the impact of Dkk and Dkk interacting protein modulating compounds or the impact of Dkk or Dkk interacting proteins on modulation of LRP5, LRP6, HBM or the Wnt pathway include the use of TaqMan PCR, conventional reverse transcriptase PCR (RT-PCR), changes in downstream surrogate markers (*i.e.*, Wnt responsive genes), and anti-Dkk Western blots for protein detection. Other methods would be readily apparent to the artisan of ordinary skill.

12. Methods to Identify Agents that Modulate at Least One Activity of Dkk, a Dkk Interacting Protein, or LRP5/LRP6/HBM

Another embodiment of the present invention provides methods for identifying agents that modulate at least one activity of Dkk, Dkk interacting proteins, and/or LRP5/LRP6/HBM proteins or preferably which specifically modulate an activity of a Dkk/Dkk interacting protein complex or an LRP5(or LRP6/HBM)/Dkk complex, or a biologically active fragment of Dkk (*e.g.*, comprising the domain which binds LRP5/LRP6/HBM) or a Dkk interacting protein complex. Such methods or assays may utilize any means of monitoring or detecting the desired activity as would be known in the art (See, *e.g.*, Wu *et al.*, *Curr. Biol.* 10:1611-4 (2000); Fedi *et al.*, *J. Biol. Chem.* 274:19465-72 (1991); Grotewold *et al.*, *Mech. Dev.* 89:151-3 (1999); Shibata *et al.*, *Mech. Dev.* 96:243-6 (2000); Wang *et al.*, *Oncogene* 19:1843-8 (2000); and Glinka *et al.*, *Nature* 391:357-62 (1998)). Potential agents which modulate Dkk include, for example, p53, the tumor suppressor protein, which can induce Dkk-1. Damage to DNA has also been observed to up-regulate Dkk-1 expression via a stabilization and activation of p53 (Wang *et al.*, *Oncogene* 19:1843-48 (2000)); and, Shou *et al.*, *Oncogene* 21:878-89 (2002)). Additionally, Fedi *et al.* (1999) purportedly showed that Dkk-1 can block the Wnt2-induced oncogenic transformation of NIH-3T3 cells.

Furthermore, it has been suggested that Dkk expression can be modulated by BMP signaling in the developing skeleton (Mukhopadhyay *et al.*, *Dev. Cell.* 1:423-34 (2001); and Grotewold *et al.*, *EMBO J.* 21:966-75 (2002)). Grotewold *et al.* additionally describe altered Dkk expression levels in response to stress signals including UV irradiation and other genotoxic stimuli. They propose that Dkk expression is pro-apoptotic. In animals expressing HBM constructs conferring high bone mass, a reduced osteoblast apoptosis effect was observed. Thus, HBM and HBM-like variants may control/alter Dkk's role in programmed cell death. Other agents which potentially modulate Dkk activity include the Dkk interacting proteins identified in Figure 5.

In one embodiment, the relative amounts of Dkk or a Dkk interacting protein of a cell population that has been exposed to the agent to be tested is compared to an unexposed control cell population. Antibodies can be used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates may be prepared from the exposed cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe, as would be known in the art. See, *e.g.*, Ed Harlow and David Lane, Antibodies: A Laboratory Manual (Cold Spring Harbor, NY, 1988) and Ed Harlow and David Lane, Using Antibodies: A Laboratory Manual (Cold Spring Harbor, NY 1998).

For example, N- and C- terminal fragments of Dkk can be expressed in bacteria and used to search for proteins which bind to these fragments. Fusion proteins, such as His-tag or GST fusion to the N- or C-terminal regions of Dkk (or to biologically active domains of Dkk-1) or a whole Dkk protein can be prepared. These fusion proteins can be coupled to, for example, Talon or Glutathione-Sepharose beads and then probed with cell lysates to identify molecules which bind to Dkk. Prior to lysis, the cells may be treated with purified Wnt proteins, RNA, or drugs which may modulate Wnt signaling or proteins that interact with downstream elements of the Wnt pathway. Lysate proteins binding to the fusion proteins can be resolved by SDS-PAGE, isolated and identified by, for example protein sequencing or mass spectroscopy, as is known in the art. See, *e.g.*, Protein Purification Applications: A Practical Approach (Simon Roe, ed., 2nd ed.

Oxford Univ. Press, 2001) and "Guide to Protein Purification" in *Meth. Enzymology* vol. 182 (Academic Press, 1997).

The activity of Dkk, a Dkk interacting protein, or a complex of Dkk with LRP5/LRP6/HBM may be affected by compounds which modulate the interaction
5 between Dkk and a Dkk interacting protein (such as those shown in Figure 5) and/or Dkk and LRP5/LRP6/HBM. The present invention provides methods and research tools for the discovery and characterization of these compounds. The interaction between Dkk and a Dkk interacting protein and/or Dkk and LRP5/6/HBM may be monitored *in vivo* and *in vitro*. Compounds which modulate the stability of a Dkk -
10 LRP5/LRP6/HBM complex are potential therapeutic compounds. Example *in vitro* methods include: Binding LRP5/6/HBM, Dkk, or a Dkk interacting protein to a sensor chip designed for an instrument such are made by Biacore (Uppsala, Sweden) for the performance of an plasmon resonance spectroscopy observation. In this method, the chip with one of Dkk, a Dkk interacting protein, or LRP5/6 is first exposed to the other
15 under conditions which permit them to form the complex. A test compound is then introduced and the output signal of the instrument provides an indication of any effect exerted by the test compound. By this method, compounds may be rapidly screened. Another, *in vitro*, method is exemplified by the SAR-by-NMR methods (Shuker *et al.*, *Science*. 274:1531-4 (1996)). Briefly, a Dkk-1 binding domain and/or LRP 5 or 6 LBD
20 are expressed and purified as ¹⁵N labeled protein by expression in labeled media. The labeled protein(s) are allowed to form the complex in solution in an NMR sample tube. The heteronuclear correlation spectrum in the presence and absence of a test compound provides data at the level of individual residues with regard to interactions with the test compound and changes at the protein-protein interface of the complex.
25 One of skill in the art knows of many other protocols, e.g. affinity capillary electrophoresis (Okun *et al. J Biol Chem* 276:1057-62 (2001); Vergun and Chu, *Methods*, 19:270-7 (1999)), fluorescence spectroscopy, electron paramagnetic resonance, etc. which can monitor the modulation of a complex and/or measure binding affinities for complex formation.

In vitro protocols for monitoring the modulation of a Dkk/LRP5/LRP6/HBM complex include the yeast two hybrid protocol. The yeast two hybrid method may be used to monitor the modulation of a complex in vivo by monitoring the expression of genes activated by the formation of a complex of fusion proteins of Dkk and LRP ligand binding domains. Nucleic acids according to the invention which encode the interacting Dkk and LRP LBD domains are incorporated into bait and prey plasmids. The Y2H protocol is performed in the presence of one or more test compounds. The modulation of the complex is observed by a change in expression of the complex activated gene. It will be appreciated by one skilled in the art that test compounds can be added to the assay directly or, in the case of proteins, can be coexpressed in the yeast with the bait and prey compounds. Similarly, fusion proteins of Dkk and Dkk interacting proteins can also be used in a Y2H screen to identify other proteins which modulate the Dkk/Dkk interacting protein complex.

Assay protocols such as these may be used in methods to screen for compounds, drugs, treatments which modulate the Dkk/Dkk interacting protein and/or Dkk/LRP5/6 complex, whether such modulation occurs by competitive binding, or by altering the structure of either LRP 5/6 or Dkk at the binding site, or by stabilizing or destabilizing the protein-protein interface. It may be anticipated that peptide aptamers may competitively bind, although induction of an altered binding site structure by steric effects is also possible.

12.1 Antibodies and Antibody Fragments

Polyclonal and monoclonal antibodies and fragments of these antibodies which bind to Dkk or LRP5/LRP6/HBM can be prepared as would be known in the art. For example, suitable host animals can be immunized using appropriate immunization protocols and the peptides, polypeptides or proteins of the invention. Peptides for use in immunization are typically about 8-40 residues long. If necessary or desired, the polypeptide immunogens can be conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), or other carrier proteins are well known in the art (See,

Harlow *et al.*, 1988). In some circumstances, direct conjugation using, for example, carbodiimide reagents, may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be desirable to provide accessibility to the polypeptide or hapten. The hapten peptides can be extended at either the amino or carboxy terminus with a cysteine residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

Anti-peptide antibodies can be generated using synthetic peptides, for example, the peptides derived from the sequence of any Dkk, including Dkk-1, or LRP5/LRP6/HBM. Synthetic peptides can be as small as 2-3 amino acids in length, but are preferably at least 3, 5, 10, or 15 or more amino acid residues long. Such peptides can be determined using programs such as DNASTar. The peptides are coupled to KLH using standard methods and can be immunized into animals such as rabbits. Polyclonal anti-Dkk or anti-LRP5/LRP6/HBM peptide antibodies can then be purified, for example using Actigel beads containing the covalently bound peptide.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known (See, e.g., Harlow *et al.*, 1988 and 1998). The immortalized cell lines secreting the desired antibodies can be screened by immunoassay in which the antigen is the peptide hapten, polypeptide or protein. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonal antibodies

which contain the immunologically significant portion can be used as agonists or antagonists of Dkk activity. Use of immunologically reactive fragments, such as the Fab, scFV, Fab', of F(ab')₂ fragments are often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of Dkk or LRP5/LRP6/HBM can also be produced in the context of chimeras with multiple species origin. Antibody reagents so created are contemplated for use diagnostically or as stimulants or inhibitors of Dkk activity.

In one embodiment, antibodies against Dkk, bind Dkk with high affinity, i.e., ranging from 10⁻⁵ to 10⁻⁹ M. Preferably, the anti-Dkk antibody will comprise a chimeric, primate, Primatized®, human or humanized antibody. Also, the invention embraces the use of antibody fragments, e.g., Fab's, Fv's, Fab's, F(ab)₂, and aggregates thereof.

Another embodiment contemplates chimeric antibodies which recognize Dkk or LRP5/LRP6/HBM. A chimeric antibody is intended to refer to an antibody with non-human variable regions and human constant regions, most typically rodent variable regions and human constant regions.

A "primatized® antibody" refers to an antibody with primate variable regions, e.g., CDR's, and human constant regions. Preferably, such primate variable regions are derived from an Old World monkey.

A "humanized antibody" refers to an antibody with substantially human framework and constant regions, and non-human complementarity-determining regions (CDRs). "Substantially" refers to the fact that humanized antibodies typically retain at least several donor framework residues (i.e., of non-human parent antibody from which CDRs are derived).

Methods for producing chimeric, primate, primatized®, humanized and human antibodies are well known in the art. See, e.g., U.S. Patent 5,530,101, issued to Queen *et al.*; U.S. Patent 5,225,539, issued to Winter *et al.*; U.S. Patents 4,816,397 and

4,816,567, issued to Boss *et al.* and Cabilly *et al.* respectively, all of which are incorporated by reference in their entirety.

The selection of human constant regions may be significant to the therapeutic efficacy of the subject anti-Dkk or LRP5/LRP6/HBM antibody. In a preferred embodiment, the subject anti-Dkk or LRP5/LRP6/HBM antibody will comprise human, gamma 1, or gamma 3 constant regions and, more preferably, human gamma 1 constant regions.

Methods for making human antibodies are also known and include, by way of example, production in SCID mice, and *in vitro* immunization.

The subject anti-Dkk or LRP5/LRP6/HBM antibodies can be administered by various routes of administration, typically parenteral. This is intended to include intravenous, intramuscular, subcutaneous, rectal, vaginal, and administration with intravenous infusion being preferred.

The anti-Dkk or LRP5/LRP6/HBM antibody will be formulated for therapeutic usage by standard methods, e.g., by addition of pharmaceutically acceptable buffers, e.g., sterile saline, sterile buffered water, propylene glycol, and combinations thereof.

Effective dosages will depend on the specific antibody, condition of the patient, age, weight, or any other treatments, among other factors. Typically effective dosages will range from about 0.001 to about 30 mg/kg body weight, more preferably from about 0.01 to 25 mg/kg body weight, and most preferably from about 0.1 to about 20 mg/kg body weight.

Such administration may be effected by various protocols, e.g., weekly, bi-weekly, or monthly, depending on the dosage administered and patient response. Also, it may be desirable to combine such administration with other treatments.

Antibodies to Dkk-1 interacting proteins, such as those identified in Figure 5, are also contemplated according to the present invention, and can be used similarly to the Dkk-1 antibodies mentioned in the above methodology.

The antibodies of the present invention can be utilized in experimental screening, as diagnostic reagents, and in therapeutic compositions.

12.2 Chemical Libraries

Agents that are assayed by these methods can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of Dkk-1 alone, Dkk-1 interacting proteins alone, or with their associated substrates, binding partners, etc. An example of randomly selected agents is the use of a chemical library or a peptide combinatorial library, or a growth broth of an organism.

The agents of the present invention can be, as examples, peptides, small molecules, vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

12.3 Peptide Synthesis

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production of polypeptides using solid phase peptide synthesis is necessitated if non-nucleic acid-encoded amino acids are to be included.

13. Uses for Agents that Modulate at Least One Activity of Dkk, a Dkk Interacting Protein, a Dkk/Dkk Interacting Protein Complex, or a Dkk/LRP5 or Dkk/LRP6 Complex

The proteins and nucleic acids of the invention, such as the proteins or polypeptides containing an amino acid sequence of LRP5, Dkk, and Dkk interacting proteins are involved in bone mass modulation and lipid modulation of other Wnt pathway mediated activity. Agents that modulate (*i.e.*, up and down-regulate) the expression of Dkk or Dkk interacting proteins, or agents, such as agonists and

antagonists respectively, of at least one activity of Dkk or a Dkk interacting protein may be used to modulate biological and pathologic processes associated with the function and activity of Dkk or a Dkk interacting protein.

As used herein, a subject can be preferably any mammal, so long as the mammal is in need of modulation of a pathological or biological process modulated by a protein of the invention. The term "mammal" means an individual belonging to the class *Mammalia*. The invention is particularly useful in the treatment of human subjects.

As used herein, a biological or pathological process modulated by Dkk or a Dkk interacting protein may include binding of Dkk to a Dkk interacting protein, Dkk to LRP5 or LRP6 or release therefrom, inhibiting or activating Dkk or a Dkk interacting protein mRNA synthesis or inhibiting Dkk or Dkk interacting protein modulated inhibition of LRP5 or LRP6 mediated Wnt signaling. Further bone-related markers may be observed such as alkaline phosphatase activity, osteocalcin production, or mineralization.

Pathological processes refer to a category of biological processes which produce a deleterious effect. For example, expression or up-regulation of expression of LRP5 or LRP6 and/or Dkk and/or a Dkk interacting protein may be associated with certain diseases or pathological conditions. As used herein, an agent is said to modulate a pathological process when the agent statistically significantly ($p < 0.05$) alters the process from its base level in the subject. For example, the agent may reduce the degree or severity of the process mediated by that protein in the subject to which the agent was administered. For instance, a disease or pathological condition may be prevented, or disease progression modulated by the administration of agents which reduce or modulate in some way the expression or at least one activity of a protein of the invention.

As LRP5/6 and Dkk are involved both directly and indirectly in bone mass modulation, one embodiment of this invention is to use Dkk or Dkk interacting protein expression as a method of diagnosing a bone condition or disease. Certain markers are associated with specific Wnt signaling conditions (e.g., *TCF/LEF* activation).

Diagnostic tests for bone conditions may include the steps of testing a sample or an

extract thereof for the presence of Dkk or Dkk interacting protein nucleic acids (*i.e.*, DNA or RNA), oligomers or fragments thereof or protein products of TCF/LEF regulated expression. For example, standard *in situ* hybridization or other imaging techniques can be utilized to observe products of Wnt signaling.

5 This invention also relates to methods of modulating bone development or bone loss conditions. Inhibition of bone loss may be achieved by inhibiting or modulating changes in the LRP5/6 mediated Wnt signaling pathway. For example, absence of LRP5 activity may be associated with low bone mass. Increased activity LRP5 may be associated with high bone mass. Therefore, modulation of LRP5 activity will in turn
10 modulate bone development. Modulation of the Dkk/LRP5/6 or Dkk/Dkk interacting protein complex via agonists and antagonists is one embodiment of a method to regulate bone development. Such modulation of bone development can result from inhibition of the activity of, for example, a Dkk/LRP(5/6) protein complex, a Dkk/Dkk interacting protein complex, upregulated transcription of the *LRP5* gene or inhibited
15 translation of Dkk or Dkk interacting protein mRNA.

 The agents of the present invention can be provided alone, or in combination with other agents that modulate a particular pathological process. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that
20 the agents will act at the same time.

 The agents of the present invention can be administered via parenteral, subcutaneous (sc), intravenous (iv), intramuscular (im), intraperitoneal (ip), transdermal or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the
25 recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

 The present invention further provides compositions containing one or more agents which modulate expression or at least one activity of a protein of the invention. While individual needs vary, determination of optimal ranges of effective amounts of
30 each component is within the skill of the art. Typical dosages of the active agent which

mediate Dkk or Dkk interacting protein activity comprise from about 0.0001 to about 50 mg/kg body weight. The preferred dosages comprise from about 0.001 to about 50 mg/kg body weight. The most preferred dosages comprise from about 0.1 to about 1 mg/kg body weight. In an average human of 70 kg, the range would be from about 7
5 µg to about 3.5 g, with a preferred range of about 0.5 mg to about 5 mg.

In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action.

10 Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, (e.g., ethyl oleate or triglycerides). Aqueous
15 injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes and other non-viral vectors can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the
20 invention may be formulated for enteral, parenteral, or topical (top) administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and
25 controlled release forms thereof.

Potentially, any compound which binds Dkk or a Dkk interacting protein or modulates the Dkk/LRP5 or Dkk/LRP6 or Dkk/Dkk interacting protein complex may be a therapeutic compound. In one embodiment of the invention, a peptide or nucleic acid aptamer according to the invention is used in a therapeutic composition. Such
30 compositions may comprise an aptamer, or a LRP5 or LRP6 fragment unmodified or

modified. In another embodiment, the therapeutic compound comprises a Dkk-1 interacting protein, or biologically active fragment thereof.

Nucleic acid aptamers have been used in compositions for example by chemical bonding to a carrier molecule such as polyethylene glycol (PEG) which may facilitate uptake or stabilize the aptamer. A di-alkylglycerol moiety attached to an RNA will embed the aptamer in liposomes, thus stabilizing the compound. Incorporating chemical substitutions (i.e. changing the 2'OH group of ribose to a 2'NH in RNA confers ribonuclease resistance) and capping, etc. can prevent breakdown. Several such techniques are discussed for RNA aptamers in Brody and Gold (*Rev. Mol. Biol.* 74:3-13 (2000)).

Peptide aptamers may be used in therapeutic applications by the introduction of an expression vector directing aptamer expression into the affected tissue such as for example by retroviral delivery, by encapsulating the DNA in a delivery complex or simply by naked DNA injection. Or, the aptamer itself or a synthetic analog may be used directly as a drug. Encapsulation in polymers and lipids may assist in delivery. The use of peptide aptamers as therapeutic and diagnostic agents is reviewed by Hoppe-Syler and Butz (*J. Mol. Med.* 78:426-430 (2000)).

In another aspect of the invention. The structure of a constrained peptide aptamer of the invention may be determined such as by NMR or X-ray crystallography. (Cavanagh et al., Protein NMR Spectroscopy: Principles and Practice, Academic Press, 1996; Drenth, Principles of Protein X-Ray Crystallography, Springer Verlag, 1999) Preferably the structure is determined in complex with the target protein. A small molecule analog is then designed according to the positions of functional elements of the 3D structure of the aptamer. (Guidebook on Molecular Modeling in Drug Design, Cohen, Ed., Academic Press, 1996; Molecular Modeling and Drug Design (Topics in Molecular and Structural Biology), Vinter and Gardner Eds., CRC Press, 1994) Thus the present invention provides a method for the design of effective and specific drugs which modulate the activity of Dkk, Dkk interacting proteins, Dkk/Dkk interacting protein complex and the Dkk/LRP complex. Small molecule mimetics of the peptide aptamers of the present invention are encompassed within the scope of the invention.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be co-administered along with other compounds typically prescribed for these conditions according to generally accepted medical practice. For example, the compounds of this invention can be administered in combination with other therapeutic agents for the treatment of bone loss. Bone loss mediating agents include bone resorption inhibitors such as bisphosphonates (e.g., alendronic acid, clodronic acid, etidronic acid, pamidronic acid, risedronic acid and tiludronic acid), vitamin D and vitamin D analogs, cathepsin K inhibitors, hormonal agents (e.g., calcitonin and estrogen), and selective estrogen receptor modulators or SERMs (e.g., raloxifene). And bone forming agents such as parathyroid hormone (PTH) and bone morphogenetic proteins (BMP).

Additionally contemplated are combinations of agents which regulate Dkk-1 and agents which regulate lipid levels such as HMG-CoA reductase inhibitors (i.e., statins such as Mevacor®, Lipitor® and other inhibitors such as Baycol®, Lescol®, Pravachol® and Zocor®), bile acid sequestrants (e.g., Colestid® and Welchol®), fibric acid derivatives (Atromid-S®, Lopid®, Tricor®), and nicotinic acid.

[0001] The compounds of this invention can be utilized *in vivo*, ordinarily in vertebrates and preferably in mammals, such as humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice, or *in vitro*.

14. Transgenic Animals

Transgenic animal models can be created which conditionally express Dkk and/or LRP5 or LRP6 and/or Dkk interacting proteins, such as those shown in Figure 5. These animals can be used as research tools for the study of the physiological effects of the Dkk-1/Dkk-1 interacting protein interaction and/or the LRP5 / Dkk interaction. Alternatively, transgenic animals can be created which express a transgenic form of Dkk alone or in addition to a transgenic form of HBM or express Dkk interacting proteins alone or in addition to a transgenic form of Dkk. Transgenic animals expressing HBM or LRP5 can be crossed with transgenic animals expressing Dkk or

Dkk interacting proteins to obtain heterozygote as well as homozygote animals which express both desired genes.

Animal models may be created to directly modulate the Dkk/Dkk interacting protein or Dkk/ LRP5 interaction activity *in vivo* to serve as a research tool for determining the efficacy of candidate compounds which modulate the Dkk/Dkk interacting protein or LRP5 / Dkk interaction activity *in vitro*. Animals, such as transgenic mice, can be created using the techniques employed to make transgenic mice that express for example, human Dkk or a Dkk interacting protein, or knockouts (KO), which may be conditional, of the gene encoding mouse Dkk or Dkk interacting protein. Knock-in animals include animals wherein genes have been introduced and animals wherein a gene that was previously knocked-out is reintroduced into the animal. Other transgenic animals can be created with inducible forms of Dkk or a Dkk interacting protein to study the effects of the gene on bone mass development and loss as well as lipid level regulation. These animals can also be used to study long term effects of Dkk or Dkk interacting protein modulation. Transgenic animals may be created to express peptide aptamers, or produce RNA aptamers. The transgenic vectors may direct expression in a tissue specific manner by the use of tissue specific promoters. In a preferred embodiment, a peptide aptamer fusion protein is expressed using a bone specific promoter. Such systems can provide a tissue specific knock-out of Dkk or Dkk interacting protein activity.

General methods for creating transgenic animals are known in the art, and are described in, for example, Strategies in Transgenic Animal Science (Glenn M. Monastersky and James M. Robl eds., ASM Press; Washington, DC, 1995); Transgenic Animal Technology: A Laboratory Handbook (Carl A. Pinkert ed., Academic Press 1994); Transgenic Animals (Louis Marie Houdebine, ed., Harwood Academic Press, 1997); Overexpression and Knockout of Cytokines in Transgenic Mice (Chaim O. Jacob, ed., Academic Press 1994); Microinjection and Transgenesis: Strategies and Protocols (Springer Lab Manual) (Angel Cid-Arregui and Alejandro Garcia-Carranca, eds., Springer Verlag 1998); and Manipulating the Mouse Embryo: A Laboratory Manual (Brigid Hogan et al., eds., Cold Spring Harbor Laboratory Press 1994).

15. Peptide and Nucleotide Aptamers and Peptide Aptamer Mimetics

Another embodiment contemplates the use of peptide and nucleotide aptamer technology to screen for agents which interact with Dkk, which block Dkk from interacting with LRP5 or LRP6, or which block any other Dkk ligand interaction, or which interact with Dkk interacting proteins, such as those shown in Figure 5. Peptide aptamers are molecules in which a variable peptide domain is displayed from a scaffold protein. Thioredoxin A (trxA) is commonly used for a scaffold. The peptide insert destroys the catalytic site of trxA. It is recognized that numerous proteins may also be used as scaffolding proteins to constrain and/or present a peptide aptamer. Other scaffold proteins that could display a constrained peptide aptamer could include staphylococcal nuclease, the protease inhibitor eglin C, the *Streptomyces tendea* alpha-amylase inhibitor Tendamistat, Sp1, and green fluorescent protein (GFP) (reviewed in Hoppe-Seyler *et al.*, *J. Steroid Biochem Mol. Biol.* 78:105-11 (2001)), and the S1 nuclease from *Staphylococcus* or M13 for phage display. Any molecule to which the aptamer could be anchored and presented in its bioactive conformation would be suitable.

Aptamers can then specifically bind to a given target protein *in vitro* and *in vivo* and have the potential to selectively block the function of their target protein. Peptide aptamers are selected from randomized expression libraries on the basis of their *in vivo* binding capacity to the desired target protein. Briefly, a target protein (e.g., Dkk, a Dkk interacting protein, or LRP5/6) is linked to a heterologous DNA binding domain (BD) and expressed as bait in a yeast test strain. Concomitantly, a library coding for different peptides (e.g., 16-mers) of randomized sequence inserted in a scaffold protein sequence, which are linked to a heterologous transcriptional activation domain (AD) is expressed as prey. If a peptide binds to a target protein, a functional transcription factor is reconstituted, in which the BD and AD are bridged together by interacting proteins. This transcription factor is then able to activate the promoter of a marker gene which can be monitored by colorimetric enzymatic assays or by growth selection. Additional variation, methods of preparing and screening methodologies are described in, for example, Hoppe-Seyler *et al.*, *J. Mol. Med.* 78: 426-430 (2000).

Nucleotide aptamers are described for example in Brody *et al.*, *Trends Mol. Biotechnol.* 74: 5-13 (2000). Additional methods of making and using nucleotide aptamers include SELEX, *i.e.*, Systematic Evolution of Ligands by Exponential Enrichment. SELEX is a process of isolating oligonucleotide ligands of a chosen target molecule (see Tuerk and Gold, *Science* 249:505-510 (1990); U.S. Pat. Nos. 5,475,096, 5,595,877, and 5,660,985). SELEX, as described in Tuerk and Gold, involves admixing the target molecule with a pool of oligonucleotides (*e.g.*, RNA) of diverse sequences; retaining complexes formed between the target and oligonucleotides; recovering the oligonucleotides bound to the target; reverse-transcribing the RNA into DNA; amplifying the DNA with polymerase chain reactions (PCR); transcribing the amplified DNA into RNA; and repeating the cycle with ever increasing binding stringency. Three enzymatic reactions are required for each cycle. It usually takes 12-15 cycles to isolate aptamers of high affinity and specificity to the target. An aptamer is an oligonucleotide that is capable of binding to an intended target substance but not other molecules under the same conditions.

In another reference, Bock *et al.*, *Nature* 355:564-566 (1990), describe a different process from the SELEX method of Tuerk and Gold in that only one enzymatic reaction is required for each cycle (*i.e.*, PCR) because the nucleic acid library in Bock's method is comprised of DNA instead of RNA. The identification and isolation of aptamers of high specificity and affinity with the method of Bock *et al.* still requires repeated cycles in a chromatographic column.

Other nucleotide aptamer methods include those described by Conrad *et al.*, *Meth. Enzymol.* 267:336-367 (1996). Conrad *et al.* describe a variety of methods for isolating aptamers, all of which employ repeated cycles to enrich target-bound ligands and require a large amount of purified target molecules. More recently described methods of making and using nucleotide aptamers include, but are not limited to those described in U.S. Patent Nos. 6,180,348; 6,051,388; 5,840,867; 5,780,610, 5,756,291 and 5,582,981.

Potentially, any compound which binds Dkk or a Dkk interacting protein or modulates the Dkk/Dkk interacting protein or Dkk/LRP5 or Dkk/LRP6 complex may be

a therapeutic compound. In one embodiment of the invention, a peptide or nucleic acid aptamer according to the invention is used in a therapeutic composition. Such compositions may comprise an aptamer, or a LRP5 or LRP6 fragment unmodified or modified.

5 Nucleic acid aptamers have been used in compositions for example by chemical bonding to a carrier molecule such as polyethylene glycol (PEG) which may facilitate uptake or stabilize the aptamer. A di-alkylglycerol moiety attached to an RNA will embed the aptamer in liposomes, thus stabilizing the compound. Incorporating chemical substitutions (*i.e.*, changing the 2'-OH group of ribose to a 2'-NH in RNA
10 confers ribonuclease resistance) and capping, etc. can prevent breakdown. Several such techniques are discussed for RNA aptamers in Brody and Gold *Rev. Mol. Biol.* 74:3-13 (2000).

Peptide aptamers may be used in therapeutic applications by the introduction of an expression vector directing aptamer expression into the affected tissue such as for
15 example by retroviral delivery, by encapsulating the DNA in a delivery complex or simple by naked DNA injection. Or, the aptamer itself or a synthetic analog may be used directly as a drug. Encapsulation in polymers and lipids may assist in delivery. The use of peptide aptamers as therapeutic and diagnostic agents is reviewed by Hoppe-Syler and Butz *J. Mol. Med.* 78:426-430 (2000).

20 In another aspect of the invention, the structure of a constrained peptide aptamer of the invention may be determined such as by NMR or X-ray crystallography. (Cavanagh et al., Protein NMR Spectroscopy : Principles and Practice, Academic Press, 1996; Drenth, Principles of Protein X-Ray Crystallography, Springer Verlag, 1999) Preferably the structure is determined in complex with the target protein. A
25 small molecule analog is then designed according to the positions of functional elements of the 3D structure of the aptamer. (Guidebook on Molecular Modeling in Drug Design, Cohen, Ed., Academic Press, 1996; Molecular Modeling and Drug Design (Topics in Molecular and Structural Biology), Vinter and Gardner Eds., CRC Press, 1994) Thus, a method is provided for the design of effective and specific drugs which
30 modulate the activity of Dkk, Dkk interacting proteins, Dkk/Dkk interacting protein

complex, and the Dkk/LRP complex. Small molecule mimics of the peptide aptamers of the present invention are also encompassed within the scope of the invention.

16. Alternative Variants of LRP5/LRP6 Having HBM Activity

A structural model of the LRP5/Zmax1 first beta-propeller module was generated based on a model prediction in Springer et al., (1998) *J. Molecular Biology*, 283:837-862. Based on the model, certain amino acid residues were identified as important variants of LRP5/HBM/Zmax1. The following three categories provide examples of such variants:

The shape of the beta-propeller resembles a disk with inward-sloping sides and a hole down the middle. Residue 171 is in a loop on the outer or top surface of the domain in blade 4 of propeller module 1. Thus, variants comprising changed residues in structurally equivalent positions in other blades; as well as residues that are slightly more interior to the binding pocket, but still accessible to the surface, are important embodiments of the present invention for the study of bone mass modulation by LRP5/HBM, for the development of pharmaceuticals and treatments of bone mass disorders, and for other objectives of the present invention. The following are examples of such variants:

A214V (a position equivalent to 171 in blade 5; alanine is not conserved in other propellers),

E128V (a position equivalent to 171 in blade 3; glutamate is not conserved in other propellers),

A65V (a position equivalent to 171 in blade 2; alanine is conserved in propellers 1-3 but not 4),

G199V (an accessible interior position in blade 5; glycine is conserved in propellers 1-3 but not 4), and

M282V (accessible interior position in blade 1; methionine is conserved in propellers 1-3 but not 4).

LRP5/Zmax1 has four beta-propeller structures; the first three beta-propeller modules conserve a glycine in the position corresponding to residue 171 in human

LRP5/Zmax1. Therefore, variants bearing a valine in the equivalent positions in the other propellers are important embodiments of the present invention. The following variants are useful for the study of bone mass modulation by LRP5/HBM, for the development of pharmaceuticals and treatments of bone mass disorders, and for other objectives of the present invention: G479V, G781V, and Q1087V.

The G171V HBM polymorphism results in "occupied space" of the beta-propeller 1, with the side-chain from the valine residue sticking out into an open binding pocket and potentially altering a ligand/protein interaction. The glycine residue is conserved in LRP5/Zmax1 propellers 1, 2 and 3 but is a glutamine in propeller 4. Therefore, the following variants of LRP5/HBM are important embodiments of the present invention for the study of bone mass modulation by LRP5/HBM, for the development of pharmaceuticals and treatments of bone mass disorders, and for other objectives of the present invention:

G171K (which introduces a charged side-chain),
G171F (which introduces a ringed side-chain),
G171I (which introduces a branched side-chain), and
G171Q (which introduces the propeller 4 residue).

Furthermore, LRP6 is the closest homolog of LRP5/Zmax1. LRP6 has a beta-propeller structure predicted to be similar, if not identical to Zmax1. The position corresponding to glycine 171 in human LRP5/Zmax1 is glycine 158 of human LRP6. Thus, corresponding variants of LRP6 are an important embodiment of the present invention for the study of the specificity of LRP5/Zmax1 versus its related family member, for the development of pharmaceuticals and treatments of bone mass disorders, and for other objectives of the present invention. Specifically, for example, a glycine to valine substitution at the structurally equivalent position, residue 158, of human LRP6 and similar variants of other species' LRP6 homologs represent important research tools.

Site-directed mutants of LRP5 were generated in the full-length human LRP5 cDNA using the QuikChange XL-Site-Directed Mutagenesis Kit (catalog #200516,

Stratagene, La Jolla, CA) following the manufacturer's protocol. The mutant sequences were introduced using complementary synthetic oligonucleotides:

A65V: TGGTCAGCGGCCTGGAGGATGTGGCCGCAGTGGACTTCC (SEQ ID NO:129) and

5 GGAAGTCCACTGCGGCCACATCCTCCAGGCCGCTGACCA (SEQ ID NO:130)

E128V: AAGCTGTACTGGACGGACTCAGTGACCAACCGCATCGAGG (SEQ ID NO:131) and

10 CCTCGATGCGGTTGGTCACTGAGTCCGTCCAGTACAGCTT (SEQ ID NO:132)

G171K: ATGTACTGGACAGACTGGAAGGAGACGCCCCGGATTGAGCG (SEQ ID NO: 133) and

CGCTCAATCCGGGGCGTCTCCTTCCAGTCTGTCCAGTACAT (SEQ ID NO:134)

15 G171F: ATGTACTGGACAGACTGGTTTGAGACGCCCCGGATTGAGCG (SEQ ID NO:135) and

CGCTCAATCCGGGGCGTCTCAAACCAGTCTGTCCAGTACAT (SEQ ID NO:136)

20 G171I: ATGTACTGGACAGACTGGATTGAGACGCCCCGGATTGAGCG (SEQ ID NO:137) and

CGCTCAATCCGGGGCGTCTCAATCCAGTCTGTCCAGTACAT (SEQ ID NO:138)

G171Q: ATGTACTGGACAGACTGGCAGGAGACGCCCCGGATTGAGCG (SEQ ID NO:139) and

25 CGCTCAATCCGGGGCGTCTCCTGCCAGTCTGTCCAGTACAT (SEQ ID NO:140)

G199V: CGGACATTTACTGGCCCAATGTACTGACCATCGACCTGGAGG (SEQ ID NO:141) and

30 CCTCCAGGTCGATGGTCAGTACATTGGGCCAGTAAATGTCCG (SEQ ID NO:142)

A214V: AGCTCTACTGGGCTGACGTCAAGCTCAGCTTCATCCACCG (SEQ ID NO: 143) and

CGGTGGATGAAGCTGAGCTTGACGTCAGCCCAGTAGAGCT (SEQ ID NO:144)

M282V: GAGTGCCCTCTACTCACCCGTGGACATCCAGGTGCTGAGCC (SEQ ID NO:145) and

GGCTCAGCACCTGGATGTCCACGGGTGAGTAGAGGGCACTC (SEQ ID NO:146)

G479V: CATGTAAGTGGACAGACTGGGTAGAGAACCCTAAAATCGAGTGTGC (SEQ ID NO:147) and

GCACACTCGATTTTAGGGTTCTCTACCCAGTCTGTCCAGTACATG (SEQ ID NO:148)

G781V: CATCTACTGGACCGAGTGGGTGCGCAAGCCGAGGATCGTGCG (SEQ ID NO:149) and

CGCACGATCCTCGGCTTGCCGACCCACTCGGTCCAGTAGATG (SEQ ID NO:150)

Q1087V: GTAAGTCACCAACATGGTGGACCGGGCAGCCAAGATCGAAGC (SEQ ID NO:151) and

CGTTGATCTTGGCTGCCCGGTCCACCATGTTGGTGAAGTAC (SEQ ID NO:152)

LRP6 G158V:

GTAAGTGGACAGACTGGGTAGAAAGTGCCAAAGATAGAACGTGC (SEQ ID NO:153) and

GCACGTTCTATCTTTGGCACTTCTACCCAGTCTGTCCAGTAC (SEQ ID NO:154).

All constructs were sequence verified to ensure that only the engineered modification was present in the gene. Once verified, each variant was functionally evaluated in the TCF-luciferase assay in U2OS cells (essentially as described in Example 7. Other functional evaluations could also be performed, such as the *Xenopus* embryo assay (essentially as described in Example 6), or other assays to evaluate Wnt

signaling, Dkk modulation, or anabolic bone effect. Binding of these mutants to Dkk, LRP-interacting proteins, Dkk-interacting proteins, or peptide aptamers to any of the preceding could also be investigated in a variety of ways such as in a two-hybrid system (such as in yeast as described in this application), or other methods.

5 Figure 24 shows the effects of the G171F mutation in propeller 1 of LRP5. This mutation is at the same position as HBM's G171V substitution. Expression of G171F results in an HBM effect. That is, in the presence of Wnt, G171F is able to activate the TCF-luciferase reporter construct. In fact, it may activate the reporter to a greater extent than either LRP5 or HBM. Furthermore, in the presence of Dkk1 and Wnt1,
10 G171F is less susceptible than LRP5 to modulation by Dkk. These data exemplify that the G171F variant modulates Wnt signaling in a manner similar to HBM. In addition, this data confirms that HBM's valine residue at 171 is not the only modification at 171 that can result in an HBM effect. Together these data support an important role for LRP5 propeller 1 in modulating Wnt pathway activity; in responding to Dkk modulation; and, in the ability to generate an HBM effect.
15

 Figure 25 shows the effects of the M282V mutation in propeller 1 of LRP5. M282 expression results in an HBM-effect. That is, in the presence of Wnt, M282 is able to activate the TCF-luciferase reporter construct. Furthermore, in the presence of Dkk1 and Wnt1, M282V is less susceptible than LRP5 to modulation by Dkk. These
20 data show that the M282V variant modulates Wnt signaling in a manner similar to HBM. In addition, this data confirms that modifications of other residues in propeller 1 of LRP5 can result in an HBM effect.

 These data support an "occupied space" model of the HBM mutation in propeller 1 and show that multiple mutations of propeller 1 are capable of generating an HBM
25 effect; the original G171V HBM mutation is not unique in this ability. Moreover, various perturbations in propeller 1 can modulate Dkk activity.

 These data illustrate the molecular mechanism of Dkk modulation of LRP signaling. Using the methods disclosed herein and in U.S. Application 60/290,071, generation of a comprehensive mutant panel will reveal residues in LRP that function in
30 Dkk modulation of Wnt signaling. Such variants of LRP5 and LRP6 that modulate Dkk

activity and the residues which distinguish them from LRP5 and LRP6 are points for therapeutic intervention by small molecule compound, antibody, peptide aptamer, or other agents. Furthermore, models of each HBM-effect mutation/polymorphism may be used in rational drug design of an HBM mimetic agent.

5 These are only a few illustrative examples presented to better describe the present invention. Variants of LRP5 which have demonstrated HBM activity in assays include G171F, M282V, G171K, G171Q and A214V. Clearly, other variants may be contemplated within the scope of the present invention. Furthermore, wherever HBM is recited in the methods of the invention, it should be understood that any such
10 alternative variant of LRP which demonstrates HBM biological activity is also encompassed by those claims.

17. Screening Assays

The two-hybrid system is extremely useful for studying protein:protein
15 interactions. See, e.g., Chien *et al.*, *Proc. Natl Acad. Sci. USA* 88:9578-82 (1991); Fields *et al.*, *Trends Genetics* 10:286-92 (1994); Harper *et al.*, *Cell* 75:805-16 (1993); Vojtek *et al.*, *Cell* 74:205-14 (1993); Luban *et al.*, *Cell* 73:1067-78 (1993); Li *et al.*, *FASEB J.* 7:957-63 (1993); Zang *et al.*, *Nature* 364:308-13 (1993); Golemis *et al.*, *Mol. Cell. Biol.* 12:3006-14 (1992); Sato *et al.*, *Proc. Natl Acad. Sci. USA* 91:9238-42 (1994);
20 Coghlan *et al.*, *Science* 267:108-111 (1995); Kalpana *et al.*, *Science* 266:2002-6 (1994); Helps *et al.*, *FEBS Lett.* 340:93-8 (1994); Yeung *et al.*, *Genes & Devel.* 8:2087-9 (1994); Durfee *et al.*, *Genes & Devel.* 7:555-569 (1993); Paetkau *et al.*, *Genes & Devel.* 8:2035-45; Spaargaren *et al.*, 1994 *Proc. Natl. Acad. Sci. USA* 91:12609-13 (1994); Ye *et al.*, *Proc. Natl Acad. Sci. USA* 91:12629-33 (1994); and U.S. Patent Nos.
25 5,989,808; 6,251,602; and 6,284,519.

Variations of the system are available for screening yeast phagemid (see, e.g., Harper, Cellular Interactions and Development: A Practical Approach, 153-179 (1993); Elledge *et al.*, *Proc. Natl Acad. Sci. USA* 88:1731-5 (1991)) or plasmid (Bartel, 1993 and Bartel, *Cell* 14:920-4 (1993)); Finley *et al.*, *Proc. Natl Acad. Sci. USA* 91:12980-4

(1994)) cDNA libraries to clone interacting proteins, as well as for studying known protein pairs.

The success of the two-hybrid system relies upon the fact that the DNA binding and polymerase activation domains of many transcription factors, such as GAL4, can be separated and then rejoined to restore functionality (Morin *et al.*, *Nuc. Acids Res.* 21:2157-63 (1993)). While these examples describe two-hybrid screens in the yeast system, it is understood that a two-hybrid screen may be conducted in other systems such as mammalian cell lines. The invention is therefore not limited to the use of a yeast two-hybrid system, but encompasses such alternative systems.

Yeast strains with integrated copies of various reporter gene cassettes, such as for example GAL.fwdarw.LacZ, GAL.fwdarw.HIS3 or GAL.fwdarw.URA3 (Bartel, in Cellular Interactions and Development: A Practical Approach, 153-179 (1993); Harper *et al.*, *Cell* 75:805-16 (1993); Fields *et al.*, *Trends Genetics* 10:286-92 (1994)) are co-transformed with two plasmids, each expressing a different fusion protein. One plasmid encodes a fusion between protein "X" and the DNA binding domain of, for example, the GAL4 yeast transcription activator (Brent *et al.*, *Cell* 43:729-36 (1985); Ma *et al.*, *Cell* 48:847-53 (1987); Keegan *et al.*, *Science* 231:699-704 (1986)), while the other plasmid encodes a fusion between protein "Y" and the RNA polymerase activation domain of GAL4 (Keegan *et al.*, 1986). The plasmids are transformed into a strain of the yeast that contains a reporter gene, such as lacZ, whose regulatory region contains GAL4 binding sites. If proteins X and Y interact, they reconstitute a functional GAL4 transcription activator protein by bringing the two GAL4 components into sufficient proximity to activate transcription. It is well understood that the role of bait and prey proteins may be alternatively switched and thus the embodiments of this invention contemplate and encompass both alternative arrangements.

Either hybrid protein alone must be unable to activate transcription of the reporter gene, the DNA-binding domain hybrid, because it does not provide an activation function, and the activation domain hybrid, because it cannot localize to the GAL4 binding sites. Interaction of the two test proteins reconstitutes the function of GAL4 and results in expression of the reporter gene. The reporter gene cassettes

consist of minimal promoters that contain the GAL4 DNA recognition site (Johnson *et al.*, *Mol. Cell. Biol.* 4:1440-8 (1984); Lorch *et al.*, *J. Mol. Biol.* 186:821-824 (1984)) cloned 5' to their TATA box. Transcription activation is scored by measuring either the expression of β -galactosidase or the growth of the transformants on minimal medium lacking the specific nutrient that permits auxotrophic selection for the transcription product, e.g., URA3 (uracil selection) or HIS3 (histidine selection). See, e.g., Bartel, 1993; Durfee *et al.*, *Genes & Devel.* 7:555-569 (1993); Fields *et al.*, *Trends Genet.* 10:286-292 (1994); and U.S. Pat. No. 5,283,173.

Generally, these methods include two proteins to be tested for interaction which are expressed as hybrids in the nucleus of a yeast cell. One of the proteins is fused to the DNA-binding domain (DBD) of a transcription factor and the other is fused to a transcription activation domain (AD). If the proteins interact, they reconstitute a functional transcription factor that activates one or more reporter genes that contain binding sites for the DBD. Exemplary two-hybrid assays which have been used for Dkk-1 or Dkk-1/LRP5 are presented in the Examples below.

Additional methods of preparing two hybrid assay systems for Dkk-1 interactors would be evident to one of ordinary skill in the art. See for example, Finley *et al.*, "Two-Hybrid Analysis of Genetic Regulatory Networks," in The Yeast Two-Hybrid System (Paul L. Bartel *et al.*, eds., Oxford, 1997); Meijia Yang, "Use of a Combinatorial Peptide Library in the Two-Hybrid Assay," in The Yeast Two-Hybrid System (Paul L. Bartel *et al.*, eds., Oxford, 1997); Gietz *et al.*, "Identification of proteins that interact with a protein of interest: Applications of the yeast two-hybrid system," *Mol. & Cell. Biochem.* 172:67-9 (1997); K. H. Young, "Yeast Two-Hybrid: So Many Interactions,(in) so Little Time," *Biol. Reprod.* 58:302-311 (1998); R. Brent *et al.*, "Understanding Gene and Allele Function with Two-Hybrid Methods," *Annu. Rev. Genet.* 31:663-704 (1997). It will be appreciated that protein networks can be elucidated by performing sequential screens of activation domain-fusion libraries.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The

following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

5

EXAMPLES

The present invention is described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well-known in the art or the techniques specifically described below were utilized:

10

For routine practice of the protocols referenced below, one of skill in the art is directed to the references cited in this application as well as the several Current Protocol guides, which are continuously updated, widely available and published by John Wiley and Sons, (New York). In the life sciences, Current Protocols publishes comprehensive manuals in Molecular Biology, Immunology, Human Genetics, Protein Science, Cytometry, Neuroscience, Pharmacology, Cell Biology, Toxicology, and

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Nucleic Acid Chemistry. Additional sources are known to one of skill in the art.

Example 1

Yeast Two Hybrid Screen Using LRP5 Ligand Binding Domain (LBD) Bait Sequences

20

In a screen against human osteoblast library (*i.e.*, HOB03C5, a custom Gibco generated Y2H compatible cDNA library from a human osteoblast cell line as described by Bodine and Komm, *Bone* 25:535-43 (1999)), an interaction with Dkk-1 was identified. The LRP5 ligand binding domain (LBD) baits used for this screen are depicted in Figures 2B and C. The basic protocol is as follows:

25

An overnight culture of the yeast strain containing the bait of interest is grown in 20 ml of appropriate selective medium containing 2% glucose at 30°C. The overnight culture is diluted by a 10 fold factor into YPDmedia supplemented with 40 mg/l of adenine, and grown for 4 hours at 30°C.

30

For each mating event, an aliquot of the frozen prey library is grown in 150 ml YAPD medium for 5 hours at 30°C.

Appropriate volumes calculated by measuring the OD600 of each culture are combined into a tube. The number of diploids to be screened is typically ten times the number of clones originally present in the prey library of interest. Assuming a mating efficiency of 20% minimum, fifty times (*i.e.*, ten times coverage multiplied by 20% mating efficiency) as many haploid cells containing the bait and as many cells containing the prey are used in any given mating event. The mixture is filtered over a 47 mm, 0.45 mm sterile Metrical filter membrane (Gelman).

Using sterile forceps, the filter is transferred onto a 100 mm² YAPD agar plate with the cell side up, removing all air bubbles underneath the filter. The plate is incubated overnight at room temperature.

The filter is transferred into a 50 ml Falcon tube using sterile forceps and 10 ml SD medium containing 2% glucose are added to resuspend the cells. The filter, once free of cells, is removed and the cell suspension is spun for 5 min. at 2,000 xg.

The cells are resuspended in 10 ml SD medium containing 2% glucose. An aliquot of 100 μ l is set aside for titration.

The cells are plated onto large square plates containing appropriate selective media and incubated at 30°C for three to five days.

To calculate the mating efficiency and to determine the total number of diploid cells screened, the 100 μ l aliquot set aside for titration is diluted and plated onto different selective media. The mating efficiency is calculated by dividing the number of diploids/ml by the lowest number of haploids/ml, either bait or prey, and multiplied by 100. For example, if 2 million diploids were obtained by mating 10 million of haploids containing a bait and 12 million of haploids containing a prey, then the mating efficiency is calculated by dividing 2 million by 10 million, which equals 0.2 and multiplied by 100 which equals 20%. Typical mating efficiencies under the above conditions are within about 20 to about 40%. The total number of diploids screened in a mating event is obtained by multiplying the number of diploids/ml by the total number of ml plated, typically about 10.

Isolation of colonies containing pairs of interacting proteins.

Yeast colonies from the interaction selection (large square) plates are picked with a sterile toothpick and patched onto plates containing the appropriate selective media and incubated at 30°C for two days.

5 To further ensure purity of the yeast, the plates are replicated onto another plate containing the same media and incubated at 30°C for another two days.

Yeast patches are scraped using a sterile toothpick and placed into a 96-well format plate containing 100 μ l SD –L –W –H with 2% glucose liquid medium.

10 Half the volume of the plate is transferred to a 96-well plate containing 50 μ l of 40% glycerol for storage. The other half is set aside for replication and galactosidase-activity assay (see below).

Cells are replicated onto a SD –L –W –H plate with 2% glucose plate to create a master plate, and incubated two days at 30°C. The master plate is replicated onto different selective media to score the strength of each interaction.

15 Cells are also replicated onto media selecting for the prey vector only for colony PCR and incubated two days at 30°C.

Galactosidase activity assay

20 Ten microliters from the 96-well plate (set aside from above) are transferred into another 96-well plate containing 100 μ l SD and 2% glucose media. The cell density is measured at OD₆₀₀ using a spectrophotometer, the OD₆₀₀ is usually between 0.03 and 0.1. Fifty microliters of Galactosidase reaction mixture (Tropix) are added to microplates (Marsh) specifically designed for the luminometer (Hewlett Packard Lumicount). Fifty microliters of the diluted cells are then added and mixed by pipetting.

25 The reaction is incubated sixty to one hundred twenty minutes at room temperature. Relative Light Units (RLUs) are read by the luminometer. Each plate contains a negative control, constituted by diploid yeast containing the bait of interest and an empty prey vector. To be scored as positive, the diploids tested have to have an RLU number at least twice as high as the negative control.

30

Example 2

Minimum interaction domain mapping

Further analysis of yeast two hybrid (Y2H) interacting proteins includes the dissection of protein motifs responsible for the interaction. Sequence alignment of multiple clones identified in the Y2H screens can help identify the smallest common region responsible for the interaction. In the absence of appropriate clones, deletion mapping of interacting domains is necessary.

PCR primers containing restriction sites suitable for cloning are designed to cover multiple sub-domains of the protein of interest (bait or prey). The methods involved in cloning, sequencing, yeast transformation, mating, and scoring of interactions are readily performed by one of ordinary skill in the art of molecular biology and genetic engineering.

Materials and Methods

Minimum interaction domain: primers were designed for PCR of the Dkk-1 clone isolated by screening a primary osteoblast cell strain (HOB03C5) library with pooled Zmax1/LRP5 ligand binding domain (LBD) baits: LBD1 (Leu969-Pro1376) and LBD4 (Arg1070-Pro1376). The primers, which are presented in 5' to 3' orientation, were as follows:

<u>SEQ ID NO</u>	<u>Primer</u>	<u>Sequence</u>
155	Forward 1	TTTTTTGTCGACCAATTCCAACGCTATCAAG
156	Forward 2	TTTTTTGTCGACCTGCGCTAGTCCCACCCGC
157	Forward 3	TTTTTTGTCGACCGTGTCTTCTGATCAAATC
158	Forward 4	TTTTTTGTCGACCGGACAAGAAGGTTCTGTTTG
159	Reverse 1	TTTTTTGCGGCCGCTTATTTGGTGTGATACATTTTGT
160	Reverse 2	TTTTTTGCGGCCGCTTAGCAAGACAGACCTTCTCC
161	Reverse 3	TTTTTTGCGGCCGCTTAGTGTCTCTGACAAGTGTG

PCR was performed using PfuTurbo® polymerase (Stratagene). The PCR products were gel purified, digested with *Sall*/*NotI* and ligated to pPC86 (Gibco/BRL) which had been linearized with *Sall*/*NotI*. Clones were recovered and sequenced to ascertain that the structure was as expected and that the Gal4 activation domain and Dkk-1 were in-frame. The ORF of Dkk-1 was Met1-His266, as in human Dkk-1 (GenBank Accession No. XM_005730).

The clones used were as follows: D5 (F1/R3: Asn34-His266), D4 (F1/R2: Asn34-Cys245), D3 (F1/R1: Asn34-Lys182), D9 (F2/R3: Cys97-His266), D12 (F3/R3, val139-His266), D14 (F4/R3: Gly183-His266), D8 (F2/R2: Cys97-Cys245), and D11 (F3/R2: Val139-Cys245). F1, F2, F3 and F4 refer respectively to Forward primers 1, 2, 3 and 4. R1, R2 and R3 refer respectively to reverse primers 1, 2 and 3.

These clones were transformed into yeast and mated with each of three yeast strains containing pDBleu (Gibco/BRL), pDBleuLBD1, and pDBleuLBD4. Positive interactions were detected by growth of the hybrids on appropriate selective media.

Results

Minimum interaction domain: Figure 6 shows that while growth was observed in diploids of D4, D5, D8, D9, and D12, no growth was observed in hybrids of D3, D11, and D12. Carboxy terminal (C-terminal) deletions indicated that while the C-terminal amino acids of Dkk-1 containing the potential N-glycosylation site (Arg246-His266) are not required for interaction with Zmax1/LRP5 LBD baits, the Cys2 domain, Gly183-Cys245, is required. N-terminal deletions also demonstrated that the region between the two cysteine domains, *i.e.* Val139 to Lys182, is also required. Two minimum interaction domain constructs were isolated: D12 (Val139-His266) and D8 (Cys97-Cys245). Similar constructs could be prepared for Dkk-1 interactors.

Example 3

Yeast-2 Hybrid screen for peptide aptamer sequences to Dkk-1

Peptide aptamer library construction

A peptide aptamer library, Tpep, was constructed, which provides a means to identify chimeric proteins that bind to a protein target (or bait) of interest using classic yeast two hybrid (Y2H) assays. The Tpep library is a combinatorial aptamer library composed of constrained random peptides, expressed within the context of the disulfide loop of *E. coli* thioredoxin (trxA), and as C-termini fusion to the *S. cerevisiae* Gal4 activation domain. The Tpep library was generated using a restriction enzyme modified recombinant Y2H prey vector, pPC86 (Gibco), which contains the trxA scaffold protein.

Generation of aptamer-encoding sequences

Aptamer-encoding sequences were produced as follows. DNA encoding random stretches of approximately sixteen amino acids surrounded by appropriate restriction sites were generated by semi-random oligonucleotide synthesis. The synthetic oligonucleotides were PCR-amplified, restriction digested, and cloned into the permissive sites within the trxA scaffold protein. The cloning strategy was to insert the random oligonucleotide sequence is in-frame with the scaffold protein coding sequence, resulting in expression of a scaffold protein-aptamer chimera. The scaffold protein is itself in-frame with the activation domain of Gal4, within the pPC86 vector that is appropriate for the aptamer to be expressed and functional in a regular Y2H assay. Additional methods of preparing aptamers would be apparent to the skilled artisan.

Generation of a permissive recombinant pPC86 vector containing the trxA coding sequence

First the *RsrII* restriction site located within the Gal4 activation domain of pPC86 (Gibco) was eliminated by site-directed mutagenesis (Quickchange™ kit, Stratagene). The amino acid sequence of the Gal4 activation domain was unchanged by this modification. The strength of different control interactions was verified to be unchanged by the modification.

Second, the *E. coli* trxA coding sequence was cloned into the *SaII* and *NotI* sites of the *RsrII*-modified pPC86. *EcoRI* and *SpeI* sites were then introduced within the trxA

*Rsr*I site. The oligonucleotides encoding the peptide aptamers were cloned into the *Eco*RI and *Spe*I sites of the resulting vector.

Example 4

Yeast-2 Hybrid screen for Dkk-1 interacting proteins

A Dkk-1 bait sequence was utilized in a yeast two hybrid screen to identify Dkk-1 interacting proteins. The procedure for the Y2H was carried out similarly to that employed in Example 1, except that the Dkk-1 bait from Figure 2C was used instead of LRP baits. The screen was performed using Hela and fetal brain libraries (Invitrogen Corporation, Carlsbad, CA). Multiple libraries were used to identify additional Dkk-1 interacting proteins and to confirm interactions found in other libraries.

The list of Dkk-1 interacting proteins uncovered in these Y2H screens are listed in Figure 5.

The interacting proteins identified in the Dkk-1 bait screen can be used in other Y2H screens with LRP baits and other Dkk-1 interacting proteins to determine more complex interactions which may modulate Dkk-1/LRP interactions and/or Wnt signaling.

Example 5

Generation of antibodies

In each of the following antibody-generating examples, the synthesis of these linear peptides is followed by injection into two New Zealand Rabbits. Subsequent boosts and bleeds are taken according to a standard ten-week protocol. The end-user receives back 5 mgs of peptide, aliquots of pre-bleeds, roughly 80 ml of crude sera from each of the two rabbits and, and ELISA titration data is obtained.

Generation of LRP5 Polymorphism-specific antibodies

Antibodies were generated to the following peptides to obtain antibodies which distinguish the HBM polymorphism versus wild-type LRP5/Zmax: MYWTDWVETPRIE

(SEQ ID NO:123) (mutant peptide) and MYWTDWGETPRIE (SEQ ID NO:124) (wild-type peptide for negative selection). Immunofluorescence data confirmed that the antibody, after affinity purification, is specific for HBM and does not recognize LRP5 (Figure 17).

Generation of LRP5 Monospecific antibodies

LRP5 monospecific polyclonal antibodies were generated to the following amino acid sequences of LRP5: Peptide 1 (a.a. 265-277) - KRTGGKRKEILSA (SEQ ID NO:125), Peptide 2 (a.a. 1178-1194) - ERVEKTTGDKRTRIQGR (SEQ ID NO:126), and Peptide 3 (a.a. 1352-1375) - KQQCDSFPDCIDGSDE (SEQ ID NO:127). Immunofluorescence confirmed that the antibody generated detects LRP5.

Generation of Dkk-1 monospecific polyclonal antibodies

Dkk-1 monospecific polyclonal antibodies were generated to the following amino acid sequences of Dkk-1: Peptide 1 (a.a. 71-85) - GNKYQTIDNYQPYPYPC (SEQ ID NO:118), Peptide 2 (a.a. 165-186) - LDGYSRRTTLSSKMYHTKGQEG (SEQ ID NO:119), Peptide 3 (a.a. 246-266) - RIQKDHHQASNSSRLHTCQRH (SEQ ID NO:120), Peptide 4 (a.a. 147-161) - RGEIETITESFGND (SEQ ID NO:121), and Peptide 5 (232-250) - EIFQRCYCGEGLSCRIQKD (SEQ ID NO:122) of human Dkk-1. Figure 26 shows the location of the various peptides selected, their relationship to the Dkk-1 amino acid sequence and polyclonal antibodies generated.

Western blots demonstrated that the antibodies generated against peptides 2 (Antibody #5521) (Figure 27) and 4 (Antibody #74397) (Figure 28) are specific toward Dkk-1. Figure 27 shows Western blots using 500 μ l of conditioned medium (CM) from non-transfected 293 cells or from 293 cells transfected with Dkk1-V5 that were immunoprecipitated by anti-V5 antibody. Bead elutes were separated by non-reducing SDS-PAGE (lanes #4, 5 of Figure 27). 20 μ l of conditioned medium from both samples (lanes #2, 3 of Figure 27) and from Dkk1-AP transfected 293 cells (lane #6 of Figure 27) were additionally separated on the gel. The Western was performed using

antibodies Anti-V5/AP (1:10,000) and Ab#5521 (10 μ g/ml). Ab#5521 detected Dkk1-V5 and Dkk1-AP from conditioned medium.

Figure 28 shows Western blot results using Ab#74397. Anti-V5/AP was tested at a 1:4000 dilution and Ab#74397 was tested at a 1:500 dilution. Ab#74397 was able to detect Dkk1-V5 in both conditioned medium and immunoprecipitated conditioned medium.

The results obtained with antibodies #5521 and #74397 are summarized in the following table:

Rabbit No.	Peptide Position	Peptide Sequence	Purified (Y/N)	Western	Immuno-precipitation	Location
5521	165-186	LDGYSR RTTLSSK MYHTKG QEG	Y (Protein G purified)	Y	N/A	Between Cy1 and Cys2 domain
74397	147-161	RGEIEETI TESFGN D	N	Y	N/A	Between Cy1 and Cys2 domain

Example 6

Effects of exogenous Dkk-1 on Wnt-mediated signaling in the *Xenopus* embryo assay

Xenopus embryos are an informative and well-established *in vivo* assay system to evaluate the modulation of Wnt signaling (McMahon *et al.*, *Cell* 58: 1075-84 (1989); Smith and Harland, 1991; reviewed in Wodarz and Nusse 1998).

Modification of the Wnt signaling pathway can be visualized by examining the embryos for a dorsalization phenotype (duplicated body axis) after RNA injection into the ventral blastomere at the 4- or 8-cell stage. On the molecular level, phenotypes can be analyzed by looking for expression of various marker genes in stage 10.5 embryos. Such markers would include general endoderm, mesoderm, and ectoderm markers as well as a variety of tissue-specific transcripts.

Analysis can be done by RT-PCR/TaqMan® and can be done on whole embryo tissue or in a more restricted fashion (microdissection). Because this system is very flexible and rapid, by injecting combinations of transcripts, such as HBM and different Wnts or Wnt antagonists, the mechanism of HBM in the Wnt pathway can thereby be dissected. Furthermore, investigations are conducted to determine whether Zmax/LRP5 and HBM differentially modulate Wnt signaling either alone, or in combination with other components. Previous studies have demonstrated that LRP6 alone or LRP5 + Wnt5a were able to induce axis duplication (dorsalization) in this system (Tamai *et al.*, *Nature* 407: 530-35 (2000)).

Constructs for Xenopus Expression (Vector pCS2⁺)

Constructs were prepared using the vector pCS2⁺. DNA inserts were subcloned in the sense orientation with respect to the vector SP6 promoter. The pCS2⁺ vector contains an SV40 virus polyadenylation signal and T3 promoter sequence (for generation of antisense mRNA) downstream of the insert.

Full length Zmax/LRP5 and HBM ORF cDNA: Insert cDNA was isolated from the full length cDNA retrovirus constructs (with optimized Kozak sequences) by *Bgl*II-*Eco*RI digestion and subcloned into the *Bam*HI-*Eco*RI sites of the pCS2⁺ vector.

Full length XWnt8: This cDNA was PCR amplified from a *Xenopus* embryo cDNA library using oligos 114484 (SEQ ID NO:162) (5'-CAGTGAATTCACCATGCAAAACACCACTTTGTTC-3') and 114487 (SEQ ID NO:163) (5'-CAGTTGCGGCCGCTCATCTCCGGTGGCCTCTG-3'). The oligos were designed to amplify the ORF with a consensus Kozak sequence at the 5' end as determined from GenBank #X57234. PCR was carried out using the following conditions: 96°C, 45 sec.; 63°C, 45 sec.; 72°C, 2 min. for 30 cycles. The resulting PCR product was purified, subcloned into pCRII-TOPO (Invitrogen Corp.), sequence verified, and digested with *Bam*HI/*Xho*I. This insert was subcloned into the vector at the *Bam*HI-*Xho*I sites.

Full length Wnt5a: A murine Wnt5a cDNA clone was purchased from Upstate Biotechnology (Lake Placid, NY) and subcloned into the *Eco*RI site of the vector.

Sequencing confirmed insert orientation.

Full length human Dkk-1: A human cDNA with GenBank accession number AF127563 was available in the public database. Using this sequence, PCR primers were designed to amplify the open reading frame with a consensus Kozak sequence immediately upstream of the initiating ATG. Oligos 117162 (SEQ ID NO:164) (5'-
5 CAATAGTCGACGAATTCACCATGGCTCTGGGCGCAGCGG-3') and 117163 (SEQ ID NO:165) (5'-GTATTGCGGCCGCTCTAGATTAGTGTCTCTGACAAGTGTGAA-3') were used to screen a human uterus cDNA library by PCR. The resulting PCR product was purified, subcloned into pCRII-TOPO (Invitrogen Corp.), sequence verified, and digested with *EcoRI/XhoI*. This insert was subcloned into the pCS2⁺ vector at the
10 *EcoRI-XhoI* sites.

Full length human Dkk-2: A full length cDNA encoding human Dkk-2 was isolated to investigate the specificity of the Zmax/LRP5/HBM interaction with the Dkk family of molecules. Dkk-1 was identified in yeast as a potential binding partner of Zmax/LRP5/HBM. Dkk-1 has also been shown in the literature to be an antagonist of
15 the Wnt signaling pathway, while Dkk-2 is not (Krupnik *et al.*, 1999). The Dkk-2 full length cDNA serves as a tool to discriminate the specificity and biological significance of Zmax/LRP5/HBM interactions with the Dkk family (e.g., Dkk-1, Dkk-2, Dkk-3, Dkk-4, Soggy, their homologs and variant, etc.). A human cDNA sequence for Dkk-2 (GenBank Accession No. NM_014421) was available in the public database. Using this
20 sequence, PCR primers were designed to amplify the open reading frame with a consensus Kozak sequence immediately upstream of the initiating ATG. Oligos 51409 (SEQ ID NO:166) (5'-CTAACGGATCCACCATGGCCGCGTTGATGCGG-3') and 51411 (SEQ ID NO:167) (5'-GATTCTGAATTCTCAAATTTCTGACACACATGG-3') were used to screen human embryo and brain cDNA libraries by PCR. The resulting
25 PCR product was purified, subcloned into pCRII-TOPO, sequence verified, and digested with *BamHI/EcoRI*. This insert was subcloned into the pCS2⁺ vector at the *BamHI-EcoRI* sites.

Full length LRP6 was isolated from the pED6dpc4 vector by *XhoI-XbaI* digestion. The full length cDNA was reassembled into the *XhoI-XbaI* sites of pCS2⁺. Insert
30 orientation was confirmed by DNA sequencing.

mRNA Synthesis and Microinjection Protocol

mRNA for microinjection into *Xenopus* embryos is generated by *in vitro* transcription using the cDNA constructs in the pCS2⁺ vector described above as template. RNA is synthesized using the Ambion mMessage mMachine high yield capped RNA transcription kit (Cat. #1340) following the manufacturer's specifications for the Sp6 polymerase reactions. RNA products were brought up to a final volume of 50 μ l in sterile, glass-distilled water and purified over Quick Spin Columns for Radiolabelled RNA Purification G50-Sephadex (Roche, Cat. #1274015) following the manufacturer's specifications. The resulting eluate was finally extracted with phenol:chloroform:isoamyl alcohol and isopropanol precipitated using standard protocols (Sambrook *et al.*, 1989). Final RNA volumes were approximately 50 μ l. RNA concentration was determined by absorbance values at 260 nm and 280 nm. RNA integrity was visualized by ethidium bromide staining of denaturing (formaldehyde) agarose gel electrophoresis (Sambrook *et al.*, 1989). Various amounts of RNA (2 pg to 1 ng) are injected into the ventral blastomere of the 4- or 8-cell *Xenopus* embryo. These protocols are described in Moon *et al.*, *Technique-J. of Methods in Cell and Mol. Biol.* 1: 76-89 (1989), and Peng, *Meth. Cell. Biol.* 36: 657-62 (1991).

Screening for Duplicated Body Axis

In vitro transcribed RNA is purified and injected into a ventral blasomere of the 4- or 8-cell *Xenopus* embryo (approx. 2 hours post-fertilization). At stage 10.5 (approx. 11 hours post-fertilization), the injected embryos are cultured for a total of 72 hours and then screened for the presence of a duplicated body axis (dorsalization) (Figure 7). Using XWnt8-injected (2-10 pg) as a positive control (Christian *et al.* (1991)) and water-injected or non-injected embryos as negative controls, we replicated the published observation that Zmax(LRP5) + Wnt5a (500 and 20 pg, respectively) could induce axis duplication. Wnt5a (20 pg) alone could not induce axis duplication (as previously reported by Moon *et al.* (1993)). We have also injected GFP RNA (100-770 pg) as a negative control to show that the amount of RNA injected is not perturbing embryo development (not shown). Strikingly, HBM + Wnt5a (500 and 20 pg, respectively)

yielded an approximately 3.5 fold more robust response of the phenotype ($p=0.043$ by Fisher's exact test) compared to Zmax(LRP5) + Wnt5a, suggesting that the HBM mutation is activating the Wnt pathway (Figures 8 and 9). The HBM/Wnt5a embryos also appear to be more "anteriorized" than the Zmax(LRP5)/Wnt5a embryos, again suggestive of a gain-of-function mutation.

The role of Dkk-1 as a modulator of Zmax/LRP5- and HBM-mediated Wnt signaling was investigated. Literature reports have previously characterized *Xenopus* and murine Dkk-1 as antagonists of the canonical Wnt pathway in the *Xenopus* system (Glinka *et al.*, *Nature* 391:357-362 (1998)). Using the human Dkk-1 construct, a dose-response assay was performed to confirm that our construct was functional and to identify the optimal amount of RNA for microinjection. Using 250 pg/embryo of hDkk-1 RNA, over 90% ($p<0.001$) of the embryos were observed to display enlarged anterior structures (big heads) as anticipated from the published reports (Figure 10).

The mechanism of hDkk-1 modulation of Wnt signaling in the presence of Zmax/LRP5 or HBM was also investigated. Without any hDkk-1 present, it was confirmed that HBM + Wnt5a was a more potent activator of Wnt signaling than Zmax/LRP5 + Wnt5a ($p<0.05$). Interestingly, in the presence of hDkk-1 (250 pg), Zmax/LRP5-mediated Wnt signaling was repressed ($p<0.05$) but hDkk-1 was unable to repress HBM-mediated Wnt signaling ($p<0.01$) (Figure 11). The specificity of this observation can be further addressed by investigating other members of the Dkk family, other Wnt genes, LRP6, additional Zmax/LRP5 mutants, and the peptide aptamers.

Example 7

Effects of exogenous Dkk and LRP5 on Wnt signaling in the TCF-luciferase Assay

Wnt activity can be antagonized by many proteins including secreted Frizzled related proteins (SFRPs), Cerberus, Wnt Inhibitory Factor-1 and Dkk-1 (Krupnik *et al.*, 1999). The Dkk family of proteins consists of Dkk-1-4 and Soggy, a Dkk-3-like protein. Dkk-1 and Dkk-4 have been shown to antagonize Wnt mediated *Xenopus* embryo development, whereas Dkk-2, Dkk-3, and Soggy do not. Unlike many of these proteins

that antagonize Wnt activity by directly interacting with Wnt proteins, Dkk-1 acts by binding to two recently identified Wnt coreceptors, LRP5 and LRP6. (Mao *et al.*, 2001; Bafico *et al.*, 2001). The details of this interaction have been examined by the present inventors and Mao *et al.* using deletion constructs of LRP6, which demonstrated that EGF repeats 3 and 4 are important for Dkk-1 interaction. Accordingly, the activity of two Dkk proteins, Dkk-1 and Dkk-2, were investigated with various Wnt members, LRP5, LRP6, and the mutant form of LRP5, designated HBM. The present invention explores whether there is any functional difference between LRP5 and HBM with regard to Dkk action on Wnt mediated signaling. Various reagents were developed, including Dkk-1 peptides, constrained LRP5 peptide aptamers, constrained Dkk-1 peptide aptamers and polyclonal antibodies to Dkk-1 (in Example 5 above) to identify factors that mimic HBM mediated Wnt signaling.

Methods

Various LRP5 constrained peptides were developed. Specifically, four peptides that interact with the LBD of LRP5 (Figure 4, constructs OST259-262 in Figure 12) and three peptides that interact with the cytoplasmic domain of LRP5 (constructs OST266-OST268 in Figure 12). In addition two Dkk-1 peptides were developed: constructs OST264 and OST265 in Figure 12, corresponding to Dkk-1 amino acids 139-266 and 96-245, containing the smallest region of Dkk-1 that interacts with LRP5 (Figure 6). The cDNA clones encoding the LRP5 LBD interacting peptides and the Dkk-1 peptides were subcloned into pcDNA3.1 with the addition of a Kozak and signal sequence to target the peptide for secretion. The constructs encoding the three peptides interacting with the cytoplasmic domain of LRP5 were also subcloned into pcDNA3.1. However, these latter constructs do not contain a signal sequence.

HOB-03-CE6 osteoblastic cells developed by Wyeth Ayerst (Philadelphia, PA) were seeded into 24-well plates at 150,000 cells per well in 1 ml of the growth media (D-MEM/F12 phenol red-free) containing 10% (v/v) heat-inactivated FBS, 1X penicillin streptomycin, and 1X Glutamax-1, and incubated overnight at 34°C. The following day, the cells were transfected using Lipofectamine 2000® (as described by the

manufacturer, Invitrogen) in OptiMEM (Invitrogen) with 0.35 μ g /well of LRP5, HBM, or control plasmid DNA (empty vector pcDNA3.1) and either Wnt1 or Wnt3a plasmid DNA. Similar experiments were performed with LRP6 plasmid DNA (0.35 μ g/well) or a control pEDdpc4 empty vector. Furthermore, each of these groups were then divided
5 into three groups, those receiving 0.35 μ g/well Dkk-1, Dkk-2, or pcDNA3.1 control DNA. All wells were transfected with 0.025 μ g/well of CMV beta-galactosidase plasmid DNA and 0.35 μ g/well 16X TCF(AS)-luciferase reporter DNA (developed by Ramesh Bhat, Wyeth-Ayerst (Philadelphia, PA)). After 4 hours of incubation, the cells were rinsed and 1 ml of fresh growth media was added to each well. The cells were cultured overnight
10 at 34°C, followed by a wash and a change of media. Cells were cultured for an additional 18-24 hours at 37°C. Cells were then lysed with 50 μ l/well of 1X lysis buffer. The extracts were assayed for beta-galactosidase activity (Galacto Reaction Buffer Diluent & Light Emission Accelerator, Tropix) using 5 μ l extract + 50 μ l beta-galactosidase diluent and luciferase activity (Luciferase Assay Reagent, Promega)
15 using 20 μ l extract.

U2OS human osteosarcoma cells were also utilized. U2OS cells (ATCC) were seeded into 96-well plates at 30,000 cells per well in 200ul of the growth media (McCoy's 5A) containing 10% (v/v) heat-inactivated FBS, 1X penicillin streptomycin, and 1X Glutamax-1, and incubated overnight at 37°C. The following day, the media
20 was replaced with OptiMEM (Invitroge) and cells were transfected using Lipofectamine 2000® (as described by the manufacturer, Invitrogen) with 0.005 μ g/well of LRP5, HBM, LRP6 or control plasmid DNA (empty vector pcDNA3.1) and either Wnt1 (.0025ug/well) or Wnt3a (.0025ug/well) plasmid DNA. In addition, the 16x-(AS) TCF-TK-firefly-luciferase (Ramesh Bhat, WHRI, Wyeth) and control TK-renilla luciferase (Promega
25 Corp.) were co-transfected at 0.3ug/well and 0.06ug/well respectively in all experiments. Furthermore, each of these groups was then divided into different groups, those receiving 0.05ug/well Dkk-1, Dkk-2, Dkk3, Dkk1-Alkaline Phosphatase (AP), mutant Dkk-1 (C220A), Soggy or pcDNA3.1 control DNA. In other experiments, cells were co-transfected with 0.005 μ g/well of LRP5, 0.0025ug/well of Wnt1 or Wnt3a (using
30 0.0025 μ g/well of a control pcDNA3.1) with LRP5-interacting aptamers (0.05ug/well).

Cells were cultured for an additional 18-20 hours at 37°C. Culture medium was removed. Cells were cultured for an additional 18-20 hours at 37°C. Culture medium was removed. Cells were then lysed with 100 μ l/well of 1X Passive Lysis Buffer (PLB) of Dual Luciferase Reagent kit (DLR-kit-Promega Corp.) 20 μ l of the lysates were
5 combined with LARII reagent of DLR-kit and assayed for TCF-firefly luciferase signal in Top Count (Packard) instrument. After measuring the Firefly readings, 100 μ l of the "Stop and Glo" reagent of DLR kit that contains a quencher and a substrate for renilla luciferase was added into each well. Immediately the renilla luciferase reading was measured using the Top Count (Packard) Instrument. The ratios of the TCF-firefly
10 luciferase to control renilla readings were calculated for each well and the mean ratio of triplicate or more wells was expressed in all data.

Results

The results of these experiments demonstrate that Dkk-1, in the presence of
15 Wnt1 and LRP5, significantly antagonized TCF-luciferase activity (Figure 14). In marked contrast, Dkk-1 had no effect on HBM/Wnt1 mediated TCF-luciferase activity (Figure 14). In similar experiments, Dkk-1 was also able to antagonize LRP5/Wnt3a but not HBM/Wnt3a mediated TCF-luciferase activity (Figure 15). These results indicate that the HBM mutation renders Dkk-1 inactive as an antagonist of Wnt1 and
20 Wnt3a signaling in HOB03CE6 osteoblastic cells. In other experiments with Wnt1, Dkk-1 had no effect on LRP5 or HBM mediated TCF-luciferase activity (Figure 14). In contrast, with either LRP5 or HBM in the presence of Wnt3a, Dkk-2 was able to antagonize the TCF-luciferase activity (Figure 15). These latter results indicate that the HBM mutation has no effect on Dkk-2 action in the presence of Wnt3a. Experiments
25 were also performed using the closely related LRP6 cDNA in HOB-03-CE6 cells. In these experiments, LRP6/Wnt1 and LRP6/Wnt3a mediated TCF-luciferase were regulated in the same manner as LRP5. Specifically, Dkk-1 antagonized LRP6/Wnt1 mediated TCF-luciferase activity, whereas Dkk-2 had no effect (Figure 14). However, similar to the action of Dkk-2 with LRP5/Wnt3a, Dkk-2 was able to antagonize
30 LRP6/Wnt3a mediated TCF-luciferase activity (Figure 15).

The results in the U2OS cells show a robust effect of the OST262 LRP5 peptide aptamer activation of Wnt signaling in the presence of Wnt3a (Figure 16). These functional results are confirmed by the results shown below in Example 11 using LRP5 peptide aptamers in the Xenopus assay. Such results affirmatively demonstrate that the effects of small molecules on LRP5/LRP6/HBM signaling can be detected using the TCF-luciferase assay.

These data demonstrate that there is a functional difference between LRP5 and HBM regarding the ability of Dkk-1 to antagonize Wnt1 and Wnt3a signaling. These data and previous data showing that Dkk-1 directly interacts with LRP5 suggests that the inability of Dkk-1 to antagonize HBM/Wnt signaling may in part contribute to the HBM phenotype. These experiments further demonstrate the ability to test various molecules (e.g., small molecules, aptamers, peptides, antibodies, LRP5 interacting proteins or Dkk-1 interacting proteins, and the like) for a LRP5 ligand that mimics HBM mediated Wnt signaling or factors that block Dkk-1 interaction with LRP5.

Example 8

Yeast-2 Hybrid Interaction Trap

Small molecule inhibitors (or partial inhibitors) of the Dkk-LRP interaction may be an excellent osteogenic therapeutic. One way to investigate this important protein-protein interaction is using Y2H techniques substantially as described above and as is well known in the art. Regions of LRP5, such as LRP5 LBD, have been found to functionally interact with Dkk. This interaction is quantitated using a reporter element known in the art, e.g., LacZ or luciferase, which is only activated when bait and prey interact. The Y2H assay is used to screen for compounds which modulate the LRP-Dkk interaction. Such a modulation would be visualized by a reduction in reporter element activation signifying a weaker or disrupted interaction, or by an enhancement of the reporter element activation signifying a stronger interaction. Thus, the Y2H assay can be used as a high-throughput screening technique to identify compounds which disrupt or enhance Dkk interaction with LRP5/LRP6/HBM, which may serve as potential therapeutics.

For example, the Interaction Trap methodology can be used as follows. The LRP5 LBD, for example, was fused with LexA and Dkk-1 was fused with either Gal4-AD or B42. With the LRP5LBD-LexA bait and the Gal4AD-Dkk prey, over a 20-fold activation of a lacZ reporter (under the control of a single LexA operator) was detected over the background. Using a Dkk-1 mutant (C220A) that is unable to bind to LRP, the interaction was reduced in yeast, showing the specificity of this interaction and system (Figure 18). As a result, small molecules may be identified that modulate this interaction between LRP and Dkk.

Example 9

Cell-Based Functional High-Throughput Assay

To develop a high throughput assay, the TCF-luciferase assay described in Example 7 was modified utilizing low level expression of endogenous LRP5/6 in U2OS and HEK293 cells. However, HOB-03-CE6 cells and any other cells which show a differential response to Dkk depending on whether LRP5, LRP6 or HBM are expressed. Using U2OS (human osteosarcoma) and HEK293 (ATCC) cells, the TCF-luciferase and tk-Renilla reporter element constructs were co-transfected along with Wnt3a/1 and Dkk. Wnt3a alone, by using endogenous LRP5/6, was able to stimulate TCF reporter gene activation. When Dkk, is co-transfected with Wnt3a/Wnt 1 and reporters (TCF-luci and tk-Renilla), Dkk represses reporter element activity. In addition, the TCF-luci signal is activated by Wnt3a/Wnt1 can be repressed by the addition of Dkk-enriched conditioned media to the cells containing Wnt3a/Wnt1 and reporters. The assay is further validated by the lack of TCF-reporter inhibition by a point mutant construct (C220A) of Dkk1.

The Dkk-mediated repression of the reporter is dependent upon the concentration of transfected Dkk cDNA or on the amount of Dkk-conditioned media added. In addition, the Dkk-mediated reporter suppression can be altered by the co-transfection of LRP5, LRP6, and HBM cDNAs in the U2OS or HEK293 cells. In general, U2OS cells show greater sensitivity to Dkk-mediated reporter suppression than that in HEK-293 cells. In U2OS cells, the transfection of LRP5/LRP6/HBM cDNA leads

to moderate activation of TCF-luci in the absence of Wnt3a/Wnt1 transfection. This activation presumably utilizes the endogenous Wnts present in U2OS cells. Under this condition, Dkk1 can repress TCF-luci and shows a differential signal between LRP5 and HBM. By co-transfecting Wnt3a/Wnt1, there is a generalized increase in the TCF-luci signal in the assay. Further, one can detect Dkk-mediated differential repression of the reporter due to LRP5 and HBM cDNA expression as well as between LRP5 and LRP6 cDNA. The repression is maximal with LRP6, moderate with LRP5, and least with HBM cDNA expression. In addition, the assay can detect the functional impact of the LRP5 interacting peptide aptamers (Figure 4), Dkk1 interacting aptamers and binding domains of Dkk-1 (Figure 6; OST264 and OST265 of Figures 12 and 13).

Using this system with a suppressed Wnt-TCF signal due to the presence of both Dkk and Wnt3a, one can screen for compounds that could alter Dkk modulation of Wnt signaling, by looking for compounds that activate or the TCF-luciferase reporter, and thereby relieve the Dkk-mediated repression of the Wnt pathway. Such compounds identified may potentially serve as HBM-mimetics and be useful, for example, as osteogenic therapeutics. Data generated from this high throughput screen are demonstrated in Figures 19-21. Figure 19 shows that Dkk1 represses Wnt3a-mediated signaling in U2OS bone cells. Figure 20 demonstrates the functional differences between LRP5, LRP6, and HBM. Dkk-1 represses LRP6 and LRP5 but has little or no effect on HBM-generated Wnt1 signaling in U2OS cells. Figure 21 demonstrates the differential effects of various Dkk family members and modified Dkks, including Dkk-1, a mutated Dkk-1 (C220A), Dkk-1-AP (modified with alkaline phosphatase), Dkk-3, and Soggy.

Example 10

DKK/LRP5/6/HBM ELISA Assay

A further method to investigate Dkk binding to LRP is via ELISA assay. Two possible permutations of this assay are exemplified. LRP5 is immobilized to a solid surface, such as a tissue culture plate well. One skilled in the art will recognize that other supports such as a nylon or nitrocellulose membrane, a silicon chip, a glass slide,

beads, etc. can be utilized. In this example, the form of LRP5 used is actually a fusion protein where the extracellular domain of LRP5 is fused to the Fc portion of human IgG. The LRP5-Fc fusion protein is produced in CHO cell extracts from stable cell lines. The LRP5-Fc fusion protein is immobilized on the solid surface via anti-human Fc antibody or by Protein-A or Protein G-coated plates, for example. The plate is then washed to remove any non-bound protein. Conditioned media containing secreted Dkk protein or secreted Dkk-epitope tagged protein (or purified Dkk or purified Dkk-epitope tagged protein) is incubated in the wells and binding of Dkk to LRP is investigated using antibodies to either Dkk or to an epitope tag. Dkk-V5 epitope tagged protein would be detected using an alkaline phosphatase tagged anti-V5 antibody.

Alternatively, the Dkk protein could be directly fused to a detection marker, such as alkaline phosphatase. Here the detection of the Dkk-LRP interaction can be directly investigated without subsequent antibody-based experiments. The bound Dkk is detected in an alkaline phosphatase assay. If the Dkk-alkaline phosphatase fusion protein is bound to the immobilized LRP5, alkaline phosphatase activity would be detected in a colorimetric readout. As a result, one can assay the ability of small molecule compounds to alter the binding of Dkk to LRP using this system. Compounds, when added with Dkk (or epitope-tagged Dkk) to each well of the plate, can be scored for their ability to modulate the interaction between Dkk and LRP based on the signal intensity of bound Dkk present in the well after a suitable incubation time and washing. The assay can be calibrated by doing cold competition experiments with unlabeled Dkk or with a second type of epitope-tagged Dkk. Any small molecule that is able to modulate the Dkk-LRP interaction may be a suitable therapeutic candidate, more preferably an osteogenic therapeutic candidate.

Example 11

Functional Evaluation of Peptide Aptamers in Xenopus

The constrained peptide aptamers constructs OST258-263 (where 258 contains the signal sequence by itself and 263 contains an irrelevant constrained peptide) (Figures 12 and 13) were used to generate RNA substantially as described in Example

7, except the vector was linearized by restriction endonuclease digestion and RNA was generated using T7 RNA polymerase.

Aptamer RNA was injected at 250 pg per blastomere using the protocol of Example 7. Wnt signaling was activated, as visualized by embryo dorsalization (duplicated body axis) with aptamers 261 and, more strongly, 262. The results of this assay are shown in Figures 22 and 23. These results suggest that aptamers 261 and 262 are able to activate Wnt signaling possibly by binding to the LBD of LRP, thereby preventing the modulation of LRP-mediated signaling by Dkk.

The aptamers of the present invention can serve as HBM-mimetics. In the *Xenopus* system they are able to induce Wnt signaling all by themselves. They may also serve as tools for rational drug design by enhancing the understanding of how peptides are able to interact with LRP and modulate Wnt signaling at the specific amino acid level. Thus, one would be able to design small molecules to mimic their effects as therapeutics. In addition, the aptamers identified as positives in this assay may be used as therapeutic molecules themselves.

Example 12

Homogenous Assay

An excellent method to investigate perturbations in protein-protein interactions is via Fluorescence Resonance Energy Transfer (FRET). FRET is a quantum mechanical process where a fluorescent molecule, the donor, transfers energy to an acceptor chromophore molecule which is in close proximity. This system has been successfully used in the literature to characterize the intermolecular interactions between LRP5 and Axin (Mao et al., *Molec. Cell Biol.* 7:801-809). There are many different fluorescent tags available for such studies and there are several ways to fluorescently tag the proteins of interest. For example, CFP (cyan fluorescent protein) and YFP (yellow fluorescent protein) can be used as donor and acceptor, respectively. Fusion proteins, with a donor and an acceptor, can be engineered, expressed, and purified.

For instance, purified LRP protein, or portions or domains thereof, fused to CFP and purified Dkk protein, or portions or domains thereof that interact with Dkk or LRP

respectively, fused to YFP can be generated and purified using standard approaches.

If LRP-CFP and Dkk-YFP are in close proximity, the transfer of energy from CFP to YFP will result in a reduction of CFP emission and an increase in YFP emission.

Energy is supplied with an excitation wavelength of 450 nm and the energy transfer is

5 recorded at emission wavelengths of 480 nm and 570 nm. The ratio of YFP emission

to CFP emission provides a gauge for changes in the interaction between LRP and

Dkk. This system is amenable for screening small molecule compounds that may alter

the Dkk-LRP protein-protein interaction. Compounds that disrupt the interaction would

be identified by a decrease in the ratio of YFP emission to CFP emission. Such

10 compounds that modulate the LRP-Dkk interaction would then be considered candidate

HBM mimetic molecules. Further characterization of the compounds can be done

using the TCF-luciferase or Xenopus embryo assays to elucidate the effects of the

compounds on Wnt signaling.

While the above example describes a cell-free, solution-phase assay using

15 purified components, a similar cell-based assay could also be performed. For example,

LRP-CFP fusion protein can be expressed in cells. The Dkk-YFP fusion protein then

could be added to the cells either as purified protein or as conditioned media. The

interaction of LRP and Dkk is then monitored as described above.

20 All references cited herein are hereby incorporated by reference in their entirety

for all purposes. The following applications are also incorporated by reference in their

entirety herein for all purposes: U.S. Application No. 60/290,071, filed May 11, 2001;

U.S. Application No. 09/544,398, filed on April 5, 2000; U.S. Application No.

09/543,771, filed April 5, 2000; 09/578,900; U.S. Application No. 09/229,319, filed

25 January 13, 1999; U.S. Provisional Application 60/071,449, filed January 13, 1998; and

International Application PCT/US00/16951, filed June 21, 2000; International PCT

Application entitled "HBM Variants That Modulate Bone Mass and Lipid Levels," filed

May 13, 2002; and International PCT Application entitled "Transgenic Animal Model of

Bone Mass Modulation," filed May 13, 2002. Additionally, this application claims priority

30 to U.S. provisional applications 60/291,311, filed May 17, 2001; 60/353,058, filed

February 1, 2002; and 60/361,293, filed March 4, 2002; the texts of which are herein incorporated by reference in their entirety for all purposes.

CLAIMS

We claim:

5 1. A method of regulating LRP5, LRP6, or HBM activity in a subject comprising administering a composition which modulates a Dkk activity in an amount effective to regulate LRP5, LRP6, or HBM activity.

10 2. The method of any of Claims 1, 24, 28, 33, 36, 37, 48, 64, 65, 93, 98, 101, 105, 107, 111, or 112, wherein the Dkk is Dkk-1.

3. The method of any of Claims 1, 24, 28, or 33, wherein the Dkk is Dkk-1 and the Dkk activity is inhibited.

15 4. The method of Claims 1 or 24, wherein the Dkk activity modulates bone mass and/or lipid levels.

5. The method of Claim 4, wherein bone mass is increased and/or lipid levels are decreased.

20 6. The method of Claim 5, wherein the increase in bone mass is determined via one or more of a decrease in fracture rate, an increase in bone strength, an increase in bone density, an increase in bone mineral density, an increase in trabecular connectivity, an increase in trabecular density, an increase in cortical density, an increase in bone diameter, and an increase in inorganic
25 bone content.

30 7. The method of any of Claims 1, 24, 28, or 33, wherein said composition comprises one or more compounds selected from the group consisting of Dkk interacting proteins, or a Dkk-binding fragment thereof.

8. The method of any of Claims 1, 24, 28, or 33, wherein said composition comprises an antisense, a siRNA, or shRNA molecule which recognizes and binds to a nucleic acid encoding one or more Dkk interacting proteins.

5

9. The method of any of Claims 1, 24, 28, or 33, and wherein said composition comprises a Dkk peptide aptamer.

10

10. The method of any of Claims 1, 24, 28, or 33, wherein said composition comprises a mimetic of a Dkk peptide aptamer.

11. The method of any of Claims 1, 24, 28, or 33, wherein said composition inhibits Dkk binding to LRP5, LRP6, or HBM.

15

12. The method of any of Claims 1, 24, 28, or 33, wherein said composition enhances binding of Dkk to LRP5, LRP6, or HBM.

20

13. The method of any of Claims 1, 24, 28, or 33, wherein said composition comprises a Dkk interacting protein peptide aptamer.

14. The method of any of Claims 1, 24, 28, or 33, wherein said composition comprises a mimetic of a Dkk interacting protein peptide aptamer.

25

15. The method of any of Claims 1, 24, 28 or 33, wherein said composition inhibits Dkk interacting protein or Dkk-binding fragment thereof binding to Dkk.

30

16. The method of any of Claims 1, 24, 28, or 33, wherein said composition enhances binding of Dkk interacting protein or Dkk-binding fragment thereof to Dkk.

17. The method of any of Claims 1, 24, 28, or 33, wherein said subject is a vertebrate or an invertebrate organism.

18. The method of any of Claims 1, 24, 28, or 33, wherein said subject
5 is a mammal.

19. The method of any of Claims 1, 24, 28, or 33, wherein said subject is a canine, a feline, an ovine, a primate, an equine, a porcine, a caprine, a camelid, an avian, a bovine, or a rodent.
10

20. The method of Claim 19, wherein said primate is a human.

21. The method of any of Claims 1, 24, 28, or 33, wherein said composition comprises an LRP5 peptide aptamer.
15

22. The method of Claim 21, wherein said peptide aptamer is OST262 (SEQ ID NO:208).

23. The method of any of Claims 1, 24, 28 or 33, wherein the
20 composition comprises an LRP5 antibody or an immunologically active fragment thereof.

24. A method of regulating Dkk-Wnt pathway activity in a subject comprising administering a composition which modulates Dkk activity in an
25 amount effective to regulate Dkk-Wnt pathway activity.

25. The method of Claims 24, 101, or 107, wherein the Wnt is one or more of Wnt1-Wnt19.

26. The method of Claim 25, wherein the Wnt is Wnt1, Wnt3, Wnt3a, or Wnt10b.

27. The method of Claim 24 wherein said composition which
5 modulates Dkk activity or modulates Dkk interaction with LRP5/LRP6/HBM is administered in an amount effective to modulate Wnt signaling.

28. A method of modulating bone mass in a subject comprising
10 administering to the subject a composition which modulates Dkk activity or Dkk interaction with LRP5, LRP6, or HBM in an amount effective to modulate bone mass in the subject.

29. The method of Claim 28, wherein bone mass is increased.

30. The method of the previous claim, wherein the increase in bone
15 mass is determined via one or more of a decrease in fracture rate, an increase in bone strength, an increase in bone density, an increase in bone mineral density, an increase in trabecular connectivity, an increase in trabecular density, an increase in cortical density, an increase in bone diameter, and an increase in
20 inorganic bone content.

31. The method of Claims 28 or 36, wherein said subject has a bone
mass disorder selected from the group consisting of a bone development
disorder, a bone fracture, age-related loss of bone, chondrodystrophy, drug-
25 induced bone disorder, high bone turnover, hypercalcemia, hyperostosis, osteogenesis imperfecta, osteomalacia, osteomyelitis, osteoporosis, Paget's disease, osteoarthritis, and rickets.

32. The method of Claim 28, wherein the composition which
30 modulates Dkk activity or Dkk interaction with LRP5, LRP6, or HBM is

administered in an amount effective to modulate the amount of trabecular and/or cortical tissue.

5 33. A method of modulating lipid levels in a subject comprising administering to the subject a composition which modulates Dkk activity or Dkk interaction with LRP5, LRP6, or HBM in an amount effective to modulate lipid levels in the subject.

10 34. The method of Claim 33, wherein lipid levels are decreased.

15 35. The method of Claim 33 or 36, wherein the subject has a lipid-modulated disorder and wherein the lipid-modulated disorder is selected from the group consisting of a cardiac condition, atherosclerosis, familial lipoprotein lipase deficiency, familial apoprotein CII deficiency, familial type 3 hyperlipoproteinemia, familial hypercholesterolemia, familial hypertriglyceridemia, multiple lipoprotein-type hyperlipidemia, elevated lipid levels due to dialysis and/or diabetes, and elevated lipid levels of unknown etiology.

20 36. A method of diagnosing low or high bone mass and/or high or low lipid levels in a subject comprising examining expression of Dkk, LRP5, LRP6, HBM, or and HBM-like variant in the subject and determining whether Dkk, LRP5, LRP6, HBM, or an HBM-like variant is over- or under-expressed to determine whether subject has (a) high or low bone mass and/or (b) has high or
25 low lipid levels.

 37. A method of screening for a compound which modulates the interaction of Dkk with LRP5, LRP6, HBM, or a Dkk-binding fragment of LRP5, LRP6, or HBM comprising:

(a) exposing Dkk and a LRP5, LRP6, and/or HBM binding fragment thereof to a compound; and

(b) determining whether said compound modulates Dkk interaction with the LRP5/LRP6/HBM binding fragment.

5

38. The method of Claim 37, wherein said modulation is determined by whether said compound binds to Dkk or the LRP5, LRP6, or HBM binding fragment thereof.

10

39. The method of Claim 37, wherein Dkk or a LRP-binding fragment thereof is attached to a substrate.

15

40. The method of Claim 37, wherein said compound comprises one or more compounds selected from the group consisting of Dkk interacting proteins, or a Dkk-binding fragment thereof.

41. The method of Claim 37 or 48, wherein said compound comprises a Dkk peptide aptamer.

20

42. The method of Claim 37 or 48, wherein said compound comprises a mimetic of a Dkk peptide aptamer.

43. The method of Claim 37 or 48, wherein said compound comprises a Dkk interacting protein peptide aptamer.

25

44. The method of Claim 37 or 48, wherein the compound comprises an LRP5 peptide aptamer.

30

45. The method of Claim 44, wherein the peptide aptamer is OST262 (SEQ ID NO:208).

46. The method of Claim 37 or 48, wherein the compound comprises an LRP5 antibody.

5 47. The method of Claim 37 or 48, wherein said compound is a mimetic of a Dkk interacting protein peptide aptamer.

48. A method of screening for a compound which modulates the interaction of Dkk with a Dkk interacting protein comprising:

10 (a) exposing a Dkk interacting protein or a Dkk-binding fragment thereof to a compound; and

(b) determining whether said compound bound to a Dkk interacting protein or the Dkk-binding fragment thereof; and

15 (c) further determining whether said compound modulates the interaction of Dkk interacting protein and Dkk.

49. The method of Claim 48, wherein the Dkk interacting protein or a Dkk-binding fragment thereof is attached to a substrate.

20 50. A composition comprising a LRP5, LRP6, or HBM activity-modulating compound and a pharmaceutically acceptable carrier therefor.

25 51. The composition of Claim 50, wherein said LRP5, LRP6, or HBM activity-modulating compound comprises a compound which binds to Dkk thereby modulating the interaction of Dkk with LRP5, LRP6, or HBM.

52. The composition of Claim 50, wherein said LRP5, LRP6, or HBM modulating compound comprises one or more Dkk interacting proteins and Dkk-binding fragments thereof.

53. The composition of Claim 50, wherein said LRP5, or LRP6, or HBM modulating compound is a monoclonal antibody or an immunologically active fragment thereof which binds to a Dkk interacting protein, or a Dkk-binding fragment thereof.

5

54. The composition of Claim 53, wherein the monoclonal antibody is human, chimeric, humanized, primatized®, or bispecific.

55. The composition of Claim 50, wherein said LRP5, LRP6, or HBM modulating compound comprises an antisense, a siRNA, or shRNA molecule which recognizes and binds to a nucleic acid encoding one or more Dkk interacting proteins.

10

56. The composition of Claim 50, wherein said LRP5, LRP6, or HBM modulating compound comprises a Dkk peptide aptamer.

15

57. The composition of Claim 50, wherein said LRP5, LRP6, or HBM modulating compound comprises a mimetic of a Dkk peptide aptamer.

20

58. The composition of Claim 50, wherein said LRP5, LRP6, or HBM modulating compound comprises a Dkk interacting protein peptide aptamer.

25

59. The composition of Claim 50, wherein said LRP5, LRP6, or HBM modulating compound comprises a mimetic of a Dkk interacting protein peptide aptamer.

60. The composition of Claim 50, wherein the compound comprises an LRP5 peptide aptamer.

61. The composition of Claim 60, wherein the peptide aptamer is OST262.

5 62. The composition of Claim 50, wherein the compound comprises an LRP5 antibody.

63. A pharmaceutical composition comprising a compound which modulates Dkk activity and a pharmaceutically acceptable carrier therefor.

10 64. A method for identifying compounds which modulate Dkk and LRP5/LRP6/HBM interactions comprising:

- (a) creating an LRP5, LRP6, or HBM fluorescent fusion protein using a first fluorescent tag; and
- (b) creating a Dkk fusion protein comprising a second fluorescent tag;
- 15 (c) adding a test compound; and
- (d) assessing changes in the ratio of fluorescent tag emissions using Fluorescence Resonance Energy Transfer (FRET) or Bioluminescence Resonance Energy Transfer (BRET) to determine whether the compound modulates Dkk and LRP5/LRP6/HBM interactions.

20 65. A method of identifying binding partners for a Dkk protein comprising the steps of:

- (a) exposing the Dkk protein(s) or a LRP5/LRP6 binding fragment thereof to a potential binding partner; and
- 25 (b) determining if the potential binding partner binds to a Dkk protein or the LRP5/LRP6 binding fragment thereof.

66. A nucleic acid encoding a Dkk interacting protein peptide aptamer comprising a nucleic acid encoding a scaffold protein in-frame with the activation

domain of Gal4 or LexA that is in-frame with a nucleic acid that encodes a Dkk interacting protein amino acid sequence.

67. A vector comprising the nucleic acid of Claim 66.

68. The nucleic acid of Claim 66, wherein the scaffold protein is trxA.

69. A method of detecting a modulatory activity of a compound on the binding interaction of a first peptide and a second peptide of a peptide binding pair that bind through extracellular interaction in their natural environment, comprising:

(i) culturing at least one eukaryotic cell comprising:

a) a nucleotide sequence encoding a first heterologous fusion protein comprising the first peptide or a segment thereof joined to a transcriptional activation protein DNA binding domain;

b) a nucleotide sequence encoding a second heterologous fusion protein comprising the second peptide or a segment thereof joined to a transcriptional activation protein transcriptional activation domain;

wherein binding of the first peptide or segment thereof and the second peptide or segment thereof reconstitutes a transcriptional activation protein; and

c) a reporter element activated under positive transcriptional control of the reconstituted transcriptional activation protein, wherein expression of the reporter element produces a selected phenotype;

(ii) incubating the eukaryotic cell in the presence of a compound under conditions suitable to detect the selected phenotype; and

- (iii) detecting the ability of the compound to affect the binding interaction of the peptide binding pair by determining whether the compound affects the expression of the reporter element which produces the selected phenotype;

5 wherein (1) said first peptide is a Dkk peptide and the second peptide is a peptide selected from LRP5, HBM, LRP6 and the Dkk-binding portion of LRP5/LRP6/HBM or (2) said first peptide is a Dkk interacting protein or the Dkk-binding fragment thereof and said second peptide is a Dkk peptide.

10 70. The method of Claim 69, wherein the eukaryotic cell is a yeast cell.

71. The method of Claim 70, wherein the yeast cell is *Saccharomyces*.

15 72. The method of Claim 71, wherein the *Saccharomyces* cell is *Saccharomyces cerevisiae*.

20 73. The method of Claim 69, wherein the Dkk is Dkk-1 and wherein the compound comprises one or more Dkk interacting proteins or a Dkk-binding fragment thereof.

74. The method of Claim 73, wherein the compound is directly added to assay.

25 75. The method of Claim 73, wherein the compound is recombinantly expressed by said eukaryotic cell in addition to said first and second peptides.

30 76. The method of Claim 69, wherein the compound comprises a Dkk peptide aptamer.

77. The method of Claim 69, wherein the compound comprises a mimetic of a Dkk peptide aptamer.

78. The method of Claim 69, wherein the compound comprises a Dkk
5 interacting protein peptide aptamer.

79. The method of Claim 69, wherein the compound comprises a mimetic of a Dkk interacting protein peptide aptamer.

80. The method of Claim 69, wherein the eukaryotic cell further
10 comprises at least one endogenous nucleotide sequence selected from the group consisting of a nucleotide sequence encoding the DNA binding domain of a transcriptional activation protein, a nucleotide sequence encoding the transcriptional activation domain of a transcriptional activation protein, and a
15 nucleotide sequence encoding the reporter element, wherein at least one of the endogenous nucleotide sequences is inactivated by mutation or deletion.

81. The method of Claim 69, wherein the peptide binding pair
20 comprises a ligand and a receptor to which the ligand binds.

82. The method of Claim 69, wherein the transcriptional activation
protein is Gal4, Gcn4, Hap1, Adr1, Swi5, Ste12, Mcm1, Yap1, Ace1, Ppr1, Arg81, Lac9, Qa1F, VP16, or a mammalian nuclear receptor.

83. The method of Claim 69, wherein at least one of the heterologous
25 fusion proteins is expressed from an autonomously-replicating plasmid.

84. The method of Claim 69, wherein the DNA binding domain is a
heterologous DNA-binding domain of a transcriptional activation protein.
30

85. The method of Claim 84, wherein the DNA binding protein is selected from the group consisting of a mammalian steroid receptor and bacterial LexA protein.

5 86. The method of Claim 69, wherein the reporter element is selected from the group consisting of *lacZ*, a polynucleotide encoding luciferase, a polynucleotide encoding green fluorescent protein (GFP), and a polynucleotide encoding chloramphenicol acetyltransferase.

10 87. The method of Claim 86, wherein the reporter element is LacZ.

88. The method of Claim 69, wherein the test sample comprises an LRP5 peptide aptamer.

15 89. The method of Claim 88, wherein the peptide aptamer is OST262 (SEQ ID NO:208).

90. The method of Claim 69, wherein the test sample comprises an LRP5 antibody.

20 91. A transgenic animal wherein Dkk-1 is knocked out in a tissue-specific fashion.

25 92. The transgenic animal of Claim 91, wherein the tissue specificity is bone tissue, cancer tissue, or liver tissue.

93. A method for identifying potential compounds which modulate Dkk activity comprising:

30 a) measuring the effect on binding of one or more Dkk interacting proteins, or a Dkk-binding fragment thereof, with Dkk or a

fragment thereof in the presence and absence of a compound;
and

b) identifying as a potential Dkk modulatory compound a
compound which modulates the binding between one or more Dkk
interacting proteins or Dkk-binding fragment thereof and Dkk or
fragment thereof.

94. A peptide aptamer of Figure 3 (SEQ ID NOs:171-188) or Figure 4
(SEQ ID NOs:189-192).

95. An antibody or antibody fragment which recognizes and binds to
one or more peptides of amino acid sequences GNKYQTIDNYQPYPYPC (SEQ ID
NO:118), LDGYSRRTTLSSKMYHTKGQEG (SEQ ID NO:119),
RIQKDHQASNSSRLHTCQRH (SEQ ID NO:120), RGEIETITESFGND (SEQ
ID NO:121), EIFQRCYCGEGLSCRIQKD (SEQ ID NO:122),
MYWTDWVETPRIE (SEQ ID NO:123), MYWTDWGETPRIE (SEQ ID NO:124),
KRTGGKRKEILSA (SEQ ID NO:125), ERVEKTTGDKRTRIQGR (SEQ ID
NO:126), KQQCDSFPDCIDGSDE (SEQ ID NO:127), or a Dkk-1 amino acid
sequence selected from the group consisting Asn34-His266 (SEQ ID NO:110),
Asn34-Cys245 (SEQ ID NO:111), Asn34-Lys182 (SEQ ID NO:112), Cys97-
His266 (SEQ ID NO:113), Val139-His266 (SEQ ID NO:114), Gly183-His266
(SEQ ID NO:115), Cys97-Cys245 (SEQ ID NO:116), or Val139-Cys245 (SEQ ID
NO:117).

96. The antibody or antibody fragment of Claim 95, wherein the
antibody is a monoclonal antibody.

97. The antibody or antibody fragment of Claim 95, wherein the
antibody is a polyclonal antibody

98. A method of identifying Dkk interacting proteins which modulate the interaction of Dkk with the Wnt signaling pathway comprising:

(a) injecting Dkk and potential Dkk interacting protein mRNA into a *Xenopus* blastomere; and

5 (b) assessing axis duplication or analyzing marker gene expression; and

(c) identifying compositions which elicit changes in axis duplication or marker gene expression as Dkk interacting proteins which modulate the interaction of Dkk with the Wnt signaling pathway.

10 99. The method of Claim 98, wherein the mRNA of HBM, LRP5/6, any Wnt, Wnt antagonist, Wnt pathway modulator, or combination of these is co-injected into the *Xenopus* blastomere.

15 100. The method of Claim 98, wherein the marker gene analyzed is Siamois, Xnr3, slug, Xbra, HNK-1, endodermin, Xlhx8, BMP2, BMP4, XLRP6, EF-1, or ODC.

20 101. A method for identifying Dkk interacting proteins which modulate the interaction of Dkk with the Wnt signaling pathway comprising:

(a) transfecting cells with constructs containing Dkk and potential Dkk interacting proteins; and

(b) assessing changes in expression of a reporter gene linked to a Wnt-responsive promoter; and

25 (c) identifying as a Dkk interacting protein any protein which alters reporter gene expression compared with cells transfected with a Dkk construct alone.

30 102. The method of Claim 101, wherein the cells are HOB-03-CE6, HEK293, or U2OS cells.

103. The method of Claim 101, wherein the Wnt-responsive promoter is TCF or LEF.

104. The method of Claim 101, wherein the cells are co-transfected with CMV β -galactosidase.

105. A method for identifying compounds which modulate Dkk and LRP5/LRP6/HBM interactions comprising:

- (a) immobilizing LRP5/LRP6/HBM to a solid surface; and
- (b) treating the solid surface with a secreted Dkk protein or a secreted epitope-tagged Dkk and a test compound; and
- (c) determining whether the compound regulates binding between Dkk and LRP5/LRP6/HBM using antibodies to Dkk or the epitope tag or by directly measuring activity of an epitope tag.

106. The method of Claim 105, wherein the epitope tag is alkaline phosphatase, histidine, or a V5 tag.

107. A method for identifying compounds which modulate the interaction of Dkk with the Wnt signaling pathway comprising:

- (a) transfecting cells with constructs containing Dkk and Wnt proteins;
- (b) assessing changes in expression of a reporter element linked to a Wnt- responsive promoter; and
- (c) identifying as a Dkk/Wnt interaction modulating compound any compound which alters reporter gene expression compared with cells transfected with a Dkk construct alone.

108. The method according to Claim 107, wherein Wnt3a and Wnt1 constructs are co-transfected into the cells.

109. The method according to Claim 107, wherein the cells are U2-OS, HOB-03-CE6, or HEK293 cells.

110. The method according to Claim 107, wherein the reporter element
5 used is TCF-luciferase, tk-Renilla, or a combination thereof.

111. A method of testing compounds that modulate Dkk-mediated activity in a mammal comprising

10 (a) providing a group of transgenic animals having (1) a regulatable one or more Dkk genes, (2) a knock-out of Dkk genes, or (3) a knock-in of one or more Dkk genes;

(b) providing a second group of control animals respectively for the group of transgenic animals in step (a); and

15 (c) exposing the transgenic animal group and control animal group to a potential Dkk-modulating compound which modulates bone mass or lipid levels; and

(d) comparing the transgenic animals and the control group of animals and determining the effect of the compound on bone mass or lipid levels in the transgenic animals as compared to the control animals.

20 112. A method of screening for compounds or compositions which modulate the interaction of Dkk and a Dkk interacting protein comprising:

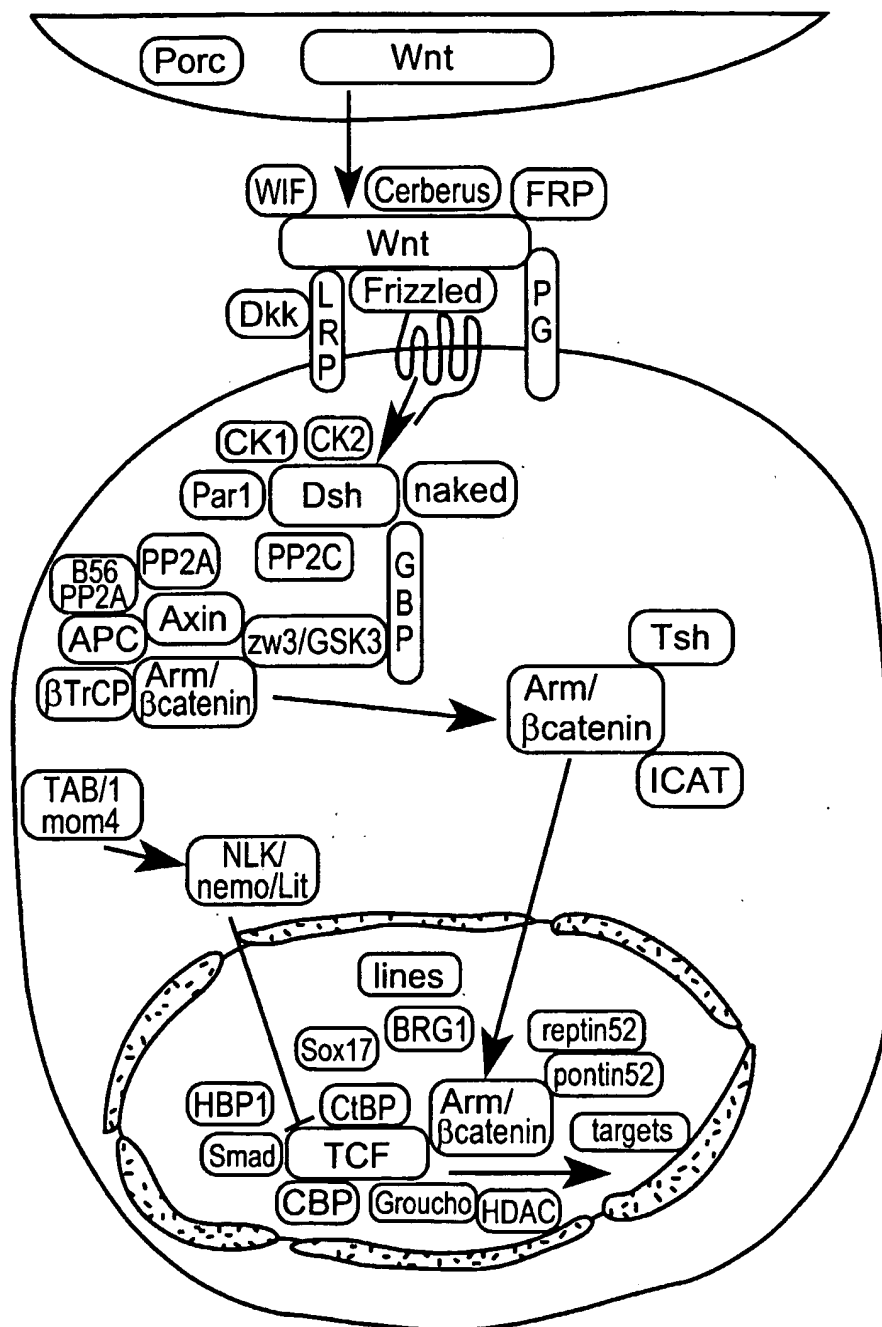
(a) exposing a Dkk interacting proteins or a Dkk-binding fragment thereof to a compound; and

25 (b) determining whether said compound binds to a Dkk interacting proteins or the Dkk-binding fragment thereof.

113. The method of Claim 112, wherein said modulation is determined by whether said compound binds to the Dkk interacting protein or the Dkk-binding fragment thereof.

5 114. An antibody or antibody fragment which recognizes and binds to a sequence depicted in Figure 3 (SEQ ID NOs:171-188) or Figure 4 (SEQ ID NOs:189-192).

1/30



Model of Wnt signaling

FIG. 1

2/30

Sequence of baits used in Y2H screens

>DKK1 (SEQ ID NO: 168)

AATTCCAACGCTATCAAGAACCTGCCCCACCGCTGGGCGGGCGCTG
CGGGGCACCCAGGCTCTGCAGTCAGCGCCGCGCCGGGAATCCTGTA
CCCGGGCGGGAATAAGTACCAGACCATTGACAACCTACCAGCCGTAC
CCGTGCGCAGAGGACGAGGAGTGCGGCACTGATGAGTACTGCGCT
AGTCCCACCCGCGGAGGGGACGCGGGCGTGCAAATCTGTCTCGCCT
GCAGGAAGCGCCGAAAACGCTGCATGCGTCACGCTATGTGCTGCC
CGGGAATTACTGCAAAAATGGAATATGTGTGTCTTCTGATCAAAAT
CATTTCCGAGGAGAAATTGAGGAAACCATCACTGAAAGCTTTGGTA
ATGATCATAGCACCTTGGATGGGTATTCCAGAAGAACCACCTTGTC
TTCAAAAATGTATCACACCAAAGGACAAGAAGGTTCTGTTTGTCTC
CGGTCATCAGACTGTGCCTCAGGATTGTGTTGTGCTAGACACTTCTG
GTCCAAGATCTGTAAACCTGTCCTGAAAGAAGGTCAAGTGTGTACC
AAGCATAGGAGAAAAGGCTCTCATGGACTAGAAATATTCCAGCGTT
GTTACTGTGGAGAAGGTCTGTCTTGCCGGATACAGAAAGATCACCA
TCAAGCCAGTAATTCTTCTAGGCTTCACACTTGTGTCAGAGACACTAA

FIG. 2A

3/30

>zmax1 LBD1 (SEQ ID NO: 169)

CTCATCCTGCCCCTGCATGGACTGAGGAACGTCAAAGCCATCGACTAT
GACCCACTGGACAAGTTCATCTACTGGGTGGATGGGCGCCAGAACATC
AAGCGAGCCAAGGACGACGGGACCCAGCCCTTTGTTTTGACCTCTCTG
AGCCAAGGCCAAAACCCAGACAGGCAGCCCCACGACCTCAGCATCGA
CATCTACAGCCGGACACTGTTCTGGACGTGCGAGGCCACCAATACCAT
CAACGTCCACAGGCTGAGCGGGGAAGCCATGGGGGTGGTGCTGCGTG
GGGACCGCGACAAGCCCAGGGCCATCGTCGTCAACGCGGAGCGAGGG
TACCTGTACTTCACCAACATGCAGGACCGGGCAGCCAAGATCGAACGC
GCAGCCCTGGACGGCACCGAGCGCGAGGTCCTCTTCACCACCGGCCTC
ATCCGCCCTGTGGCCCTGGTGGTAGACAACACACTGGGCAAGCTGTTC
TGGGTGGACGCGGACCTGAAGCGCATTGAGAGCTGTGACCTGTCAGG
GGCCAACCGCCTGACCCTGGAGGACGCCAACATCGTGCAGCCTCTGGG
CCTGACCATCCTTGGCAAGCATCTCTACTGGATCGACCGCCAGCAGCA
GATGATCGAGCGTGTGGAGAAGACCACCGGGGACAAGCGGACTCGCA
TCCAGGGCCGTGTCGCCCACCTCACTGGCATCCATGCAGTGGAGGAAG
TCAGCCTGGAGGAGTTCTCAGCCCACCCATGTGCCCCGTGACAATGGTG
GCTGCTCCACATCTGTATTGCCAAGGGTGATGGGACACCACGGTGCT
CATGCCCAGTCCACCTCGTGCTCCTGCAGAACCTGCTGACCTGTGGAG
AGCCGCCCACCTGCTCCCCGGACCAAGTTTGCATGTGCCACAGGGGAGA
TCGACTGTATCCCCGGGGCCTGGCGCTGTGACGGCTTTCCCGAGTGCG
ATGACCAGAGCGACGAGGAGGGCTGCCCCGTGTGCTCCGCCGCCAGT
TCCCCTGCGCGCGGGGTCAAGTGTGTGGACCTGCGCCTGCGCTGCGACG
GCGAGGCAGACTGTCAGGACCGCTCAGACGAGGCGGACTGTGACGCC
ATCTGCCTGCCCAACCAGTTCCGGTGTGCGAGCGGCCAGTGTGTCCTC
ATCAAACAGCAGTGCGACTCCTTCCCCGACTGTATCGACGGCTCCGAC
GAGCTCATGTGTGAAATCACCAAGCCGCCC

FIG. 2B

4/30

>zmax1 LBD4 (SEQ ID NO: 170)

AGGGCCATCGTCGTCAACGCGGAGCGAGGGTACCTGTACTTCACCAA
CATGCAGGACCGGGCAGCCAAGATCGAACGCGCAGCCCTGGACGGCA
CCGAGCGCGAGGTCCTCTTCACCACCGGCCTCATCCGCCCTGTGGCCC
TGGTGGTAGACAACACACTGGGGCAAGCTGTTCTGGGTGGACGCGGAC
CTGAAGCGCATTGAGAGCTGTGACCTGTCAGGGGCCAACCGCCTGAC
CCTGGAGGACGCCAACATCGTGCAGCCTCTGGGCCTGACCATCCTTGG
CAAGCATCTCTACTGGATCGACCGCCAGCAGCAGATGATCGAGCGTG
TGGAGAAGACCACCGGGGACAAGCGGACTCGCATCCAGGGGCCGTGTC
GCCCCACCTCACTGGCATCCATGCAGTGGAGGAAGTCAGCCTGGAGGA
GTTCTCAGCCCACCCATGTGCCCCGTGACAATGGTGGCTGCTCCCACAT
CTGTATTGCCAAGGGTGATGGGACACCACGGTGCTCATGCCCAGTCCA
CCTCGTGCTCCTGCAGAACCTGCTGACCTGTGGAGAGCCGCCACCTG
CTCCCCGGACCAGTTTGCATGTGCCACAGGGGAGATCGACTGTATCCC
CGGGGCCTGGCGCTGTGACGGCTTTCCCGAGTGCGATGACCAGAGCG
ACGAGGAGGGCTGCCCCGTGTGCTCCGCCGCCAGTTCCCCTGCGCGC
GGGGTCAGTGTGTGGACCTGCGCCTGCGCTGCGACGGCGAGGCAGAC
TGTCAGGACCGCTCAGACGAGGCGGACTGTGACGCCATCTGCCTGCC
CAACCAGTTCCGGTGTGCGAGCGGCCAGTGTGTCCTCATCAAACAGC
AGTGCGACTCCTTCCCCGACTGTATCGACGGCTCCGACGAGCTCATGT
GTGAAATCACCAAGCCGCCCTAAGCGGCCGC

FIG. 2C

5/30

Screen of DKK1 X
Peptide Library

name	motif	# hits	SEQ ID NO:
252-1	SVGCLLCAGLGVWSLS	3	171
252-2	WCCCGLFRGVCVWSCGAD D	2	172
252-3	GWRRCDWCGCVSWCWV	1	173
252-4	MPGSVSHCWGGICEAL	8	174
252-15	SCCAVDVCLRCGGWFR	1	175
252-16	SVLGTCCCCGGWILCE	2	176
252-17	VLSVCEVCGGVFVRRRC	1	177
252-18	GMWYWSGRDCALCWL	1	178
252-19	CTAVMWGVGSAVAYLGE	1	179
252-20	WCWWCGCRGVVWR	1	180
252-21	CVCASFCCVCGLRLL	1	181
252-23	TYEVCEECCGGRVRMWV	6	182
252-25	VVVCASCGQVWHGSGA	2	183
252-26	CCRCCHCWDCEWHMCV	1	184
252-27	FCASCCWCGCDCFGWV	2	185
252-32	CDYCWSCGVWCPSSWL	3	186
252-47	VYLCVWCGAARFGCYG	1	187
252-48	FCVCGCCWCWCAACWC	1	188

FIG. 3

peptide #	peptide seq	# hits	SEQ ID NO:
9	VVLCSRCGRLWRWSCG	1	189
12	EVRQVTCIRCRRGFL	1	190
13	GGGGMWEAWSCYACG	1	191
14	GWRWCGRCGALWWRRV	3	192

FIG. 4

6/30

Gene	Genbank Accession #	Protein Accession #
granulin	M75161	AAA58617
similar to cys/His rich protein	BC004544	AAH04544
IGF-BINDING PROTEIN 6	M69054	AAA88070
latent TGFb binding protein 4	AF051344	AAC39879
NOTCH 2	AF315356	AAG37073
fibulin 1	X53743	CAA37772
MDC15 (ADAM15)	U46005	AAC51112
DKFZp761G02121(notch1 Ca++ binding like)	AL137311	CAB70690
chordin	AF076612	AAC69835
fibronectin 1	U42594	AAD00019
MG50(melanoma associated antigen)	AF200348	AAF06354
unknown (notch 4-like)	AX068260	CAC27245
Slit 1	AB017167	BAA35184
tomoregulin (agarin repeat homology)	AB004064	BAA90820
sprouty 1	AF041037	AAC39566
sprouty 2	AF039843	AAC04258
NOV1	X96584	CAA65403
agrin	AF016903	AAC39776
fibrillin 1	L13923	AAB02036
thrombospondin1	X04665	CAA28370
ADAM19	AF134707	AAF22162
Nafl alpha	AJ011895	CAA09855
laminin alpha 5	Z95636	CAB09137
CRIM1	AF167706	AAF34409
nidogen	M30269	AAA59932
fibulin-2	X82494	CAA57876
thrombospondin 2	L12350	AAA03703
KIAA1323	AB037744	BAA92561
fibrillin-2	U03272	AAA18950
MEGF9	AB011542	BAA32470
integrin beta 1	X07979	CAA30790
matrilin-2 precursor	U69263	AAC51260
tenascin	X56160	A32160

FIG. 5

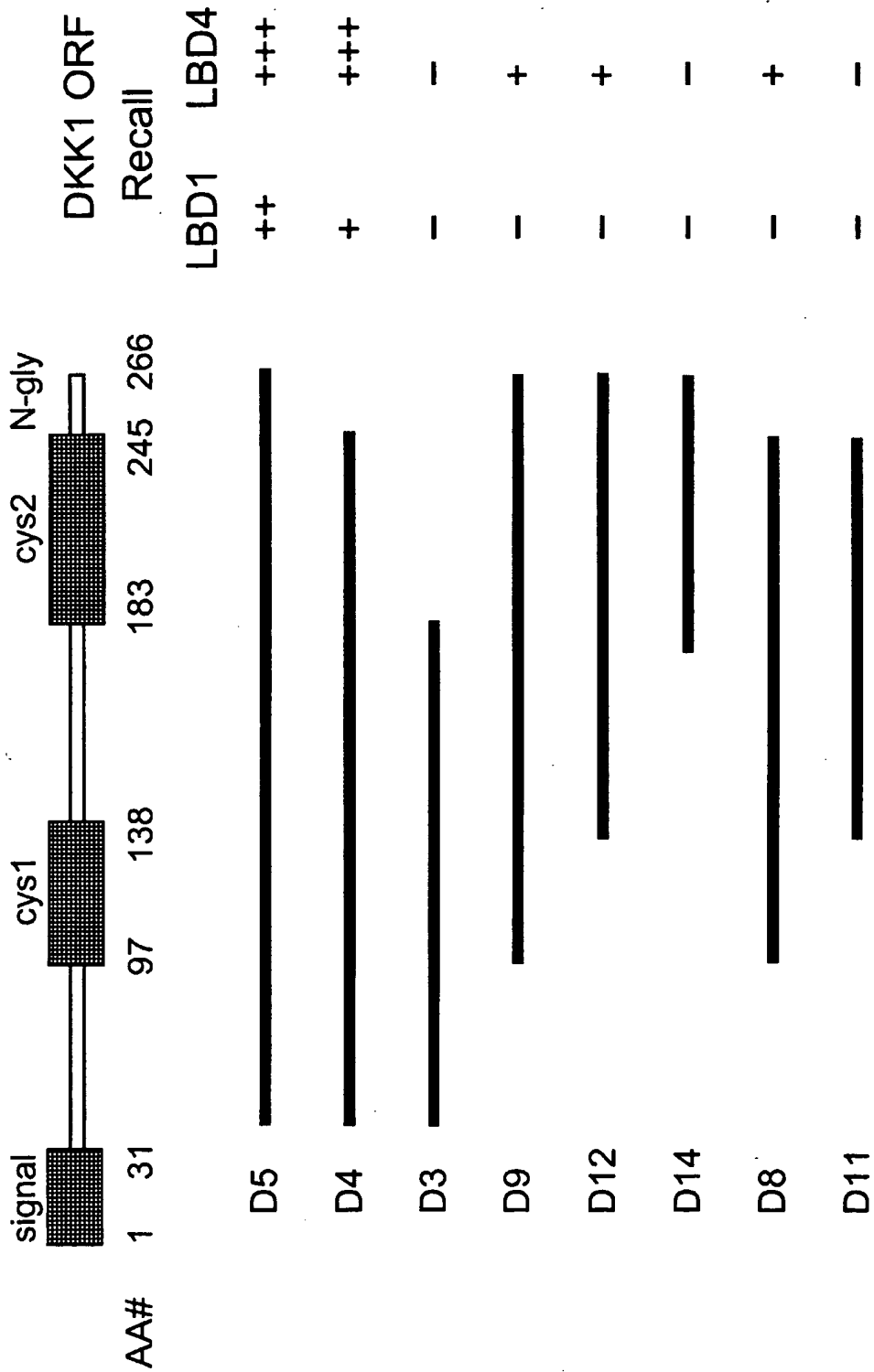


FIG. 6

8/30

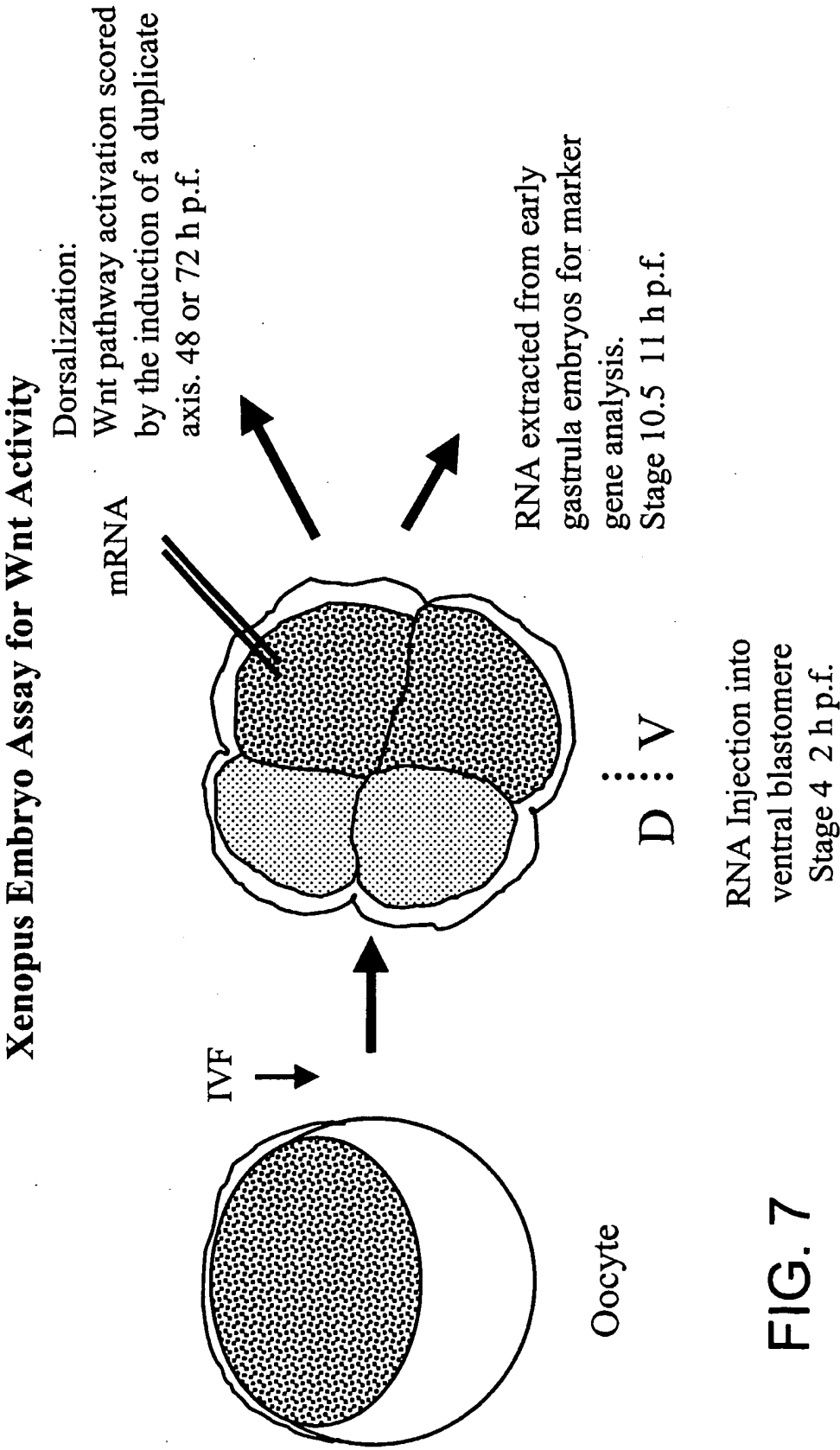


FIG. 7

9/30

In the Presence of Wnt5a, HBM1 is More Potent than
Zmax as a Stimulator of Wnt Signaling

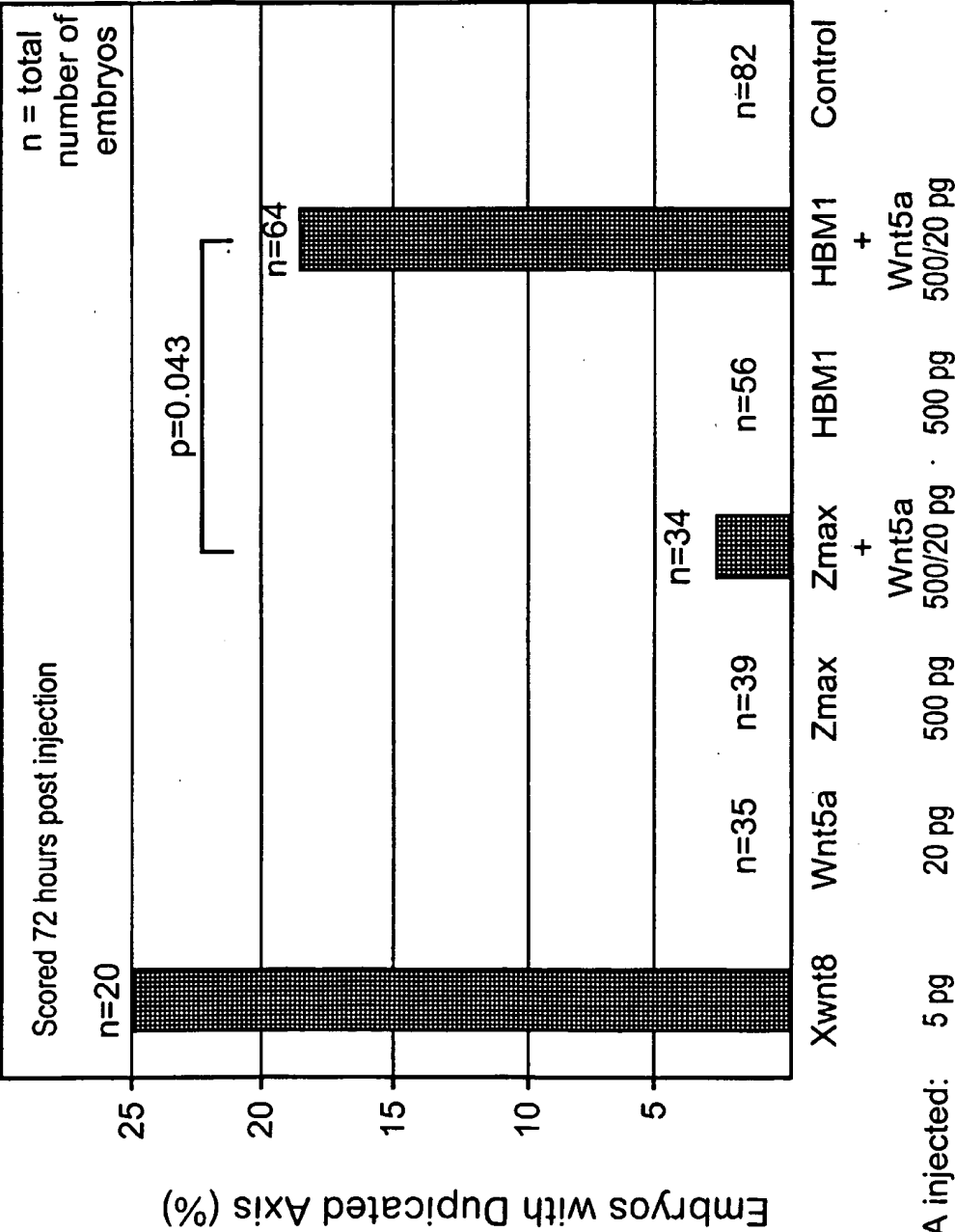


FIG. 8

10/30

Both Zmax and HBM1, in the presence of Wnt5a, induce secondary axis formation in Xenopus (photos at 48 hrs post-injection)

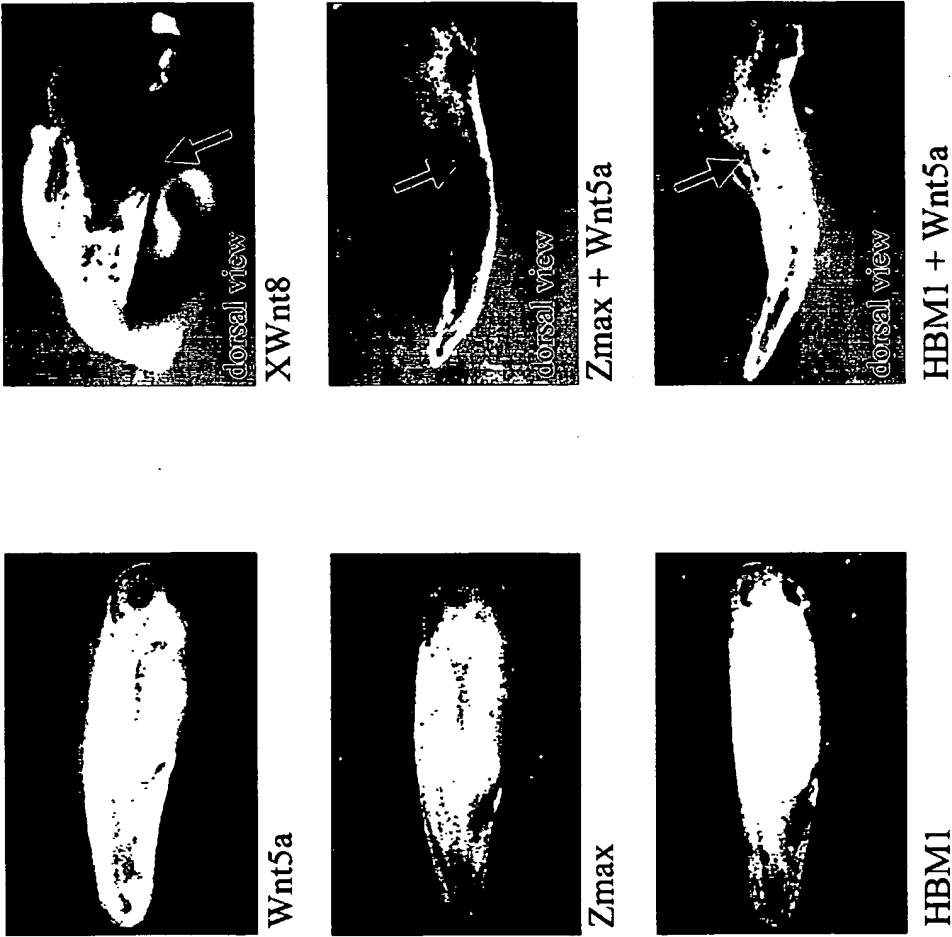


FIG. 9

11/30

Human Dkk-1 Represses the Canonical Wnt Pathway

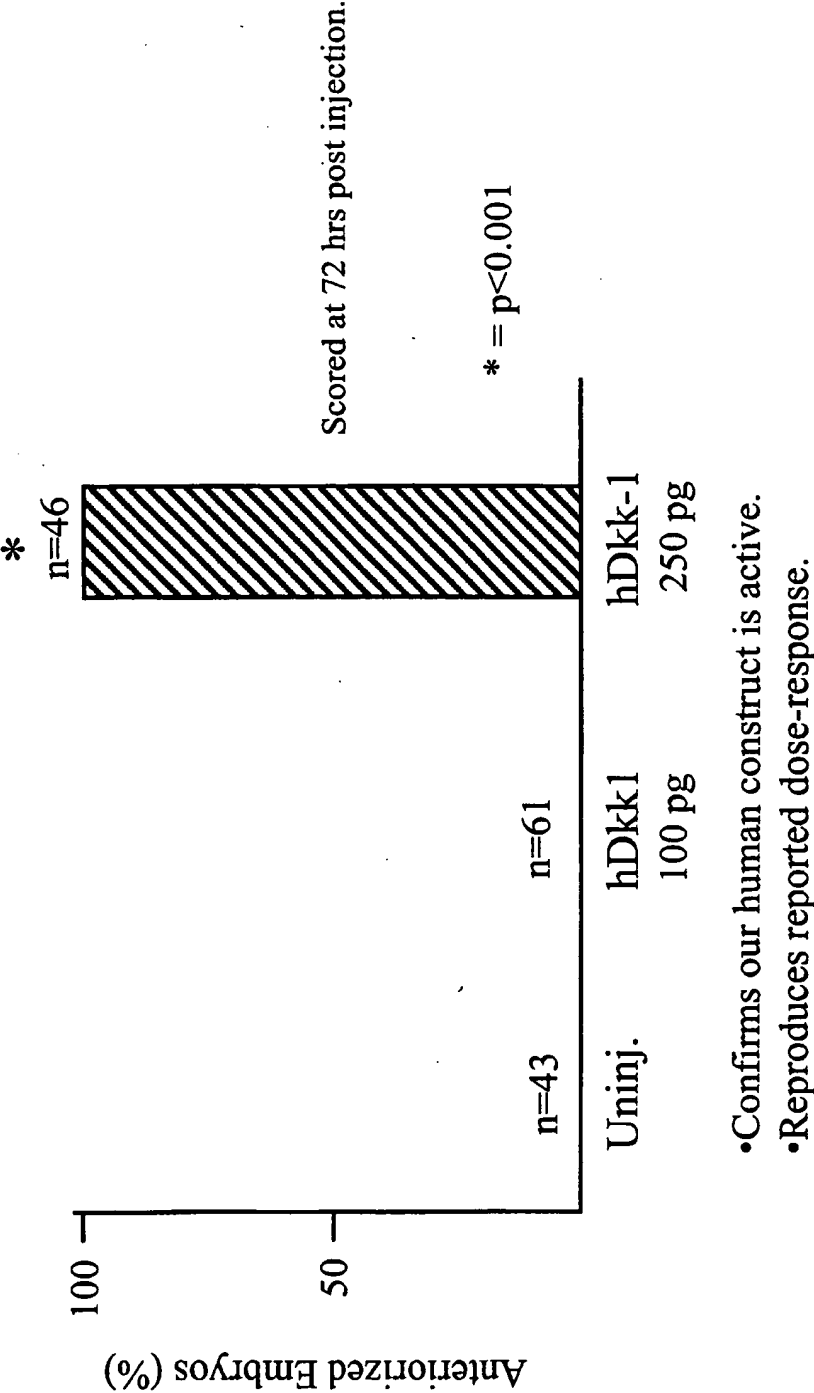


FIG. 10

hDkk1 Represses Zmax- but Not HBM1-Mediated Wnt Signaling

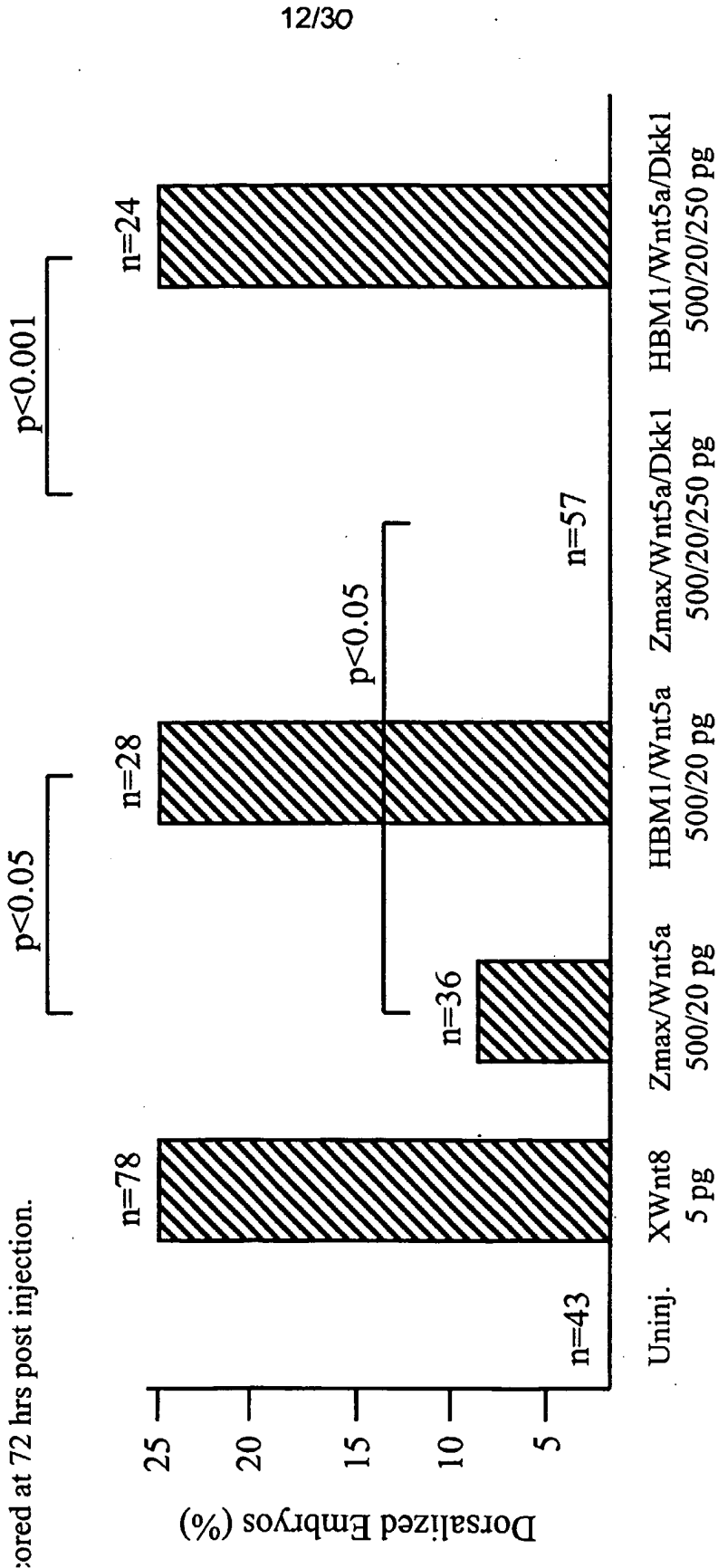


FIG. 11

Listed are the pcDNA3.1 construct names followed by the DNA sequence

OST258 (control for OST 259-OST262 and OST264, OST265)

AAGCTTGCCACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACGG
ATCC

OST259 (SEQ ID NO: 193)

AAGCTTGCCACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACGG
ATCCATGAGCGATAAAATTATTACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCC
TCGTCGATTTCTGGGCAGAGTGGTGCGGTCCGAATTCCGTTGGTTCTGTGTTCCGCTTGTGGGCGTTTGTGGCGGTGG
TCGTGTGGGACTAGTGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGCTGACGAATATCAGGGCAAAC
GACCGTTGCAAACTGAACATCGATCAAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACTCTGC
TGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGTTCTCTCGAC
GCTAACCTGGCGTAAGCGGCCGC

OST260 (SEQ ID NO: 194)

AAGCTTGCCACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACGG
ATCCATGAGCGATAAAATTATTACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCC
TCGTCGATTTCTGGGCAGAGTGGTGCGGTCCGAATTCGGGTGGCGGTGGTGTGGTGGTGGTGGTGGTGGTGGTGGTGG
CGGCGTGTACTAGTGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGCTGACGAATATCAGGGCAAAC
GACCGTTGCAAACTGAACATCGATCAAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACTCTGC
TGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGTTCTCTCGAC
GCTAACCTGGCGTAAGCGGCCGC

OST261 (SEQ ID NO: 195)

AAGCTTGCCACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACGG
ATCCATGAGCGATAAAATTATTACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCC
TCGTCGATTTCTGGGCAGAGTGGTGCGGTCCGAATTCGGGTGGCGGTGGTGTGGTGGTGGTGGTGGTGGTGGTGGTGG
TTTCTGTTGACTAGTGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGCTGACGAATATCAGGGCAAAC
GACCGTTGCAAACTGAACATCGATCAAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACTCTGC
TGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGTTCTCTCGAC
GCTAACCTGGCGTAAGCGGCCGC

OST262 (SEQ ID NO: 196)

AAGCTTGCCACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACGG
ATCCATGAGCGATAAAATTATTACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCC
TCGTCGATTTCTGGGCAGAGTGGTGCGGTCCGAATTCGGGTGGGGGGATGATTGGGAGGCTTGGAGTTGTTAT
GCGTGTGGGACTAGTGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGCTGACGAATATCAGGGCAAAC
GACCGTTGCAAACTGAACATCGATCAAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACTCTGC
TGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGTTCTCTCGAC
GCTAACCTGGCGTAAGCGGCCGC

OST263 (SEQ ID NO: 197)

AAGCTTGCCACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGACGG
ATCCATGAGCGATAAAATTATTACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCC
TCGTCGATTTCTGGGCAGAGTGGTGCGGTCCGAATTCCTTGTGGATTGGGCGGGTGATCAGGGTCTGTTTCGGCGT
TTTGTCTTTTACTAGTGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGCTGACGAATATCAGGGCAAAC
GACCGTTGCAAACTGAACATCGATCAAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACTCTGC
TGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGTTCTCTCGAC
GCTAACCTGGCGTAAGCGGCCGC

FIGURE 12A

OST264 (SEQ ID NO: 198)

AAGCTTGGCCACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCAGGTTCCACTGGTGACGG
ATCCGTGTCTTCTGATCAAAATCATTTCCGAGGAGAAATTGAGGAAACCATCCTGAAAGCTTTGGTAATGATCATA
GCACCTTGGATGGGTATTCCAGAAGAACCACCTTGTCTTCAAAATGTATCACACCAAAGGACAAGAAGGTTCTGTT
TGCTCCGGTCATCAGACTGTGCCTCAGGATTGTGTTGTGCTAGACACTTCTGGTCCAAGATCTGTAAACCTGTCCT
GAAAGAAGGTCAAGTGTGTACCAAGCATAGGAGAAAAGGCTCTCATGGACTAGAAATATTCCAGCGTTGTTACTGTG
GAGAAGGTCTGTCTTGGCCGATACAGAAAGATCACCATCAAGCCAGTAATTCTTCTAGGCTTCACACTTGTGAGAGA
CACTAAGCGGCCGC

OST265 (SEQ ID NO: 199)

AAGCTTGGCCACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCAGGTTCCACTGGTGACGG
ATCCTGCGCTAGTCCCACCCGCGAGGGGACGCGGGCGTGCAAAATCTGTCTCGCCTGCAGGAAGCGCCGAAAACGCT
GCATGCGTCACGCTATGTGCTGCCCGGGAATTACTGCAAAATGGAATATGTGTCTTCTGATCAAAATCATTTT
CGAGGAGAAATGAGGAAACCATCACTGAAAGCTTTGGTAATGATCATAGCACCTTGGATGGGTATTCCAGAAGAAC
CACCTTGTCTTCAAAATGTATCACACCAAAGGACAAGAAGGTTCTGTTTGTCTCCGGTCATCAGACTGTGCCTCAG
GATTGTGTTGTGCTAGACACTTCTGGTCCAAGATCTGTAAACCTGTCTGAAAGAAGGTCAAGTGTGTACCAAGCAT
AGGAGAAAAGGCTCTCATGGACTAGAAATATTCCAGCGTTGTTACTGTGGAGAAGGTCTGTCTTGCTAAGCGGCCGC

OST266 (SEQ ID NO: 200)

AAGCTTGGCCACCATGGGCGATAAAATTATTCACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGG
GGCGATCCTCGTCGATTTCTGGGCAGAGTGGTGCGGTCCGAATTCCTATGCGTGGTGTGTTTCTGTAGTAGGTGTA
GGTGGTGGTTGGCTTGGACTAGTGGTCCGTGCAAAATGATCGCCCGATTCTGGATGAAATCGCTGACGAATATCAG
GGCAAACTGACCGTTGCAAACTGAACATCGATCAAAACCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCC
GACTCTGCTGCTGTTCAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGT
TCCTCGACGCTAACCTGGCGTAAGCGGCCGC

OST267 (SEQ ID NO: 201)

AAGCTTGGCCACCATGGGCGATAAAATTATTCACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGG
GGCGATCCTCGTCGATTTCTGGGCAGAGTGGTGCGGTCCGAATTCCTATGAGGTTGTGAGGTTGTGGAGTCCGT
ATCCTTGGTCTTGGGTGACTAGTGGTCCGTGCAAAATGATCGCCCGATTCTGGATGAAATCGCTGACGAATATCAG
GGCAAACTGACCGTTGCAAACTGAACATCGATCAAAACCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCC
GACTCTGCTGCTGTTCAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGT
TCCTCGACGCTAACCTGGCGTAAGCGGCCGC

OST268 (SEQ ID NO: 202)

AAGCTTGGCCACCATGGGCGATAAAATTATTCACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGG
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CTGAGGCGGGTAGGTTTTATTGTAAGTGGTCCGTGCAAAATGATCGCCCGATTCTGGATGAAATCGCTGACGAA
TATCAGGGCAAACTGACCGTTGCAAACTGAACATCGATCAAAACCTGGCACTGCGCCGAAATATGGCATCCGTGG
TATCCCGACTCTGCTGCTGTTCAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGA
AAGAGTTCTCGACGCTAACCTGGCGTAAGCGGCCGC

OST269 (SEQ ID NO: 203)

(irrelevant control peptide for OST266-OST268)

AAGCTTGGCCACCATGGGCGATAAAATTATTCACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGG
GGCGATCCTCGTCGATTTCTGGGCAGAGTGGTGCGGTCCGAATTCCTTGTGGATTGGGCCGGGTGATCAGGGTCTGT
TTCGGCGTTTTGTTTTTACTAGTGGTCCGTGCAAAATGATCGCCCGATTCTGGATGAAATCGCTGACGAATATCAG
GGCAAACTGACCGTTGCAAACTGAACATCGATCAAAACCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCC
GACTCTGCTGCTGTTCAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTGAAAGAGT
TCCTCGACGCTAACCTGGCGTAAGCGGCCGC

FIGURE 12B

Listed below are the amino acid sequences corresponding to the pcDNA3.1 constructs in Appendix 1A

OST258

METDTLLLWVLLLWVPGSTGDGS

OST259 (SEQ ID NO: 204)

METDTLLLWVLLLWVPGSTGDGSM SDKIIHLTDDSFDTDLVKADGAILVDFWAEWCGPNSVVLCSRCGRLWRWSCGT
SGPCKMIAPILDEIADEYQGKLTVAKLNIDQNP GTAPKYGIRGIPTLLL FKNGEVAATKVGALSKGQLKEFLDANLA

OST260 (SEQ ID NO: 205)

METDTLLLWVLLLWVPGSTGDGSM SDKIIHLTDDSFDTDLVKADGAILVDFWAEWCGPNSGWRWCGRCGALWRRVT
SGPCKMIAPILDEIADEYQGKLTVAKLNIDQNP GTAPKYGIRGIPTLLL FKNGEVAATKVGALSKGQLKEFLDANLA

OST261 (SEQ ID NO: 206)

METDTLLLWVLLLWVPGSTGDGSM SDKIIHLTDDSFDTDLVKADGAILVDFWAEWCGPNSEVRQVTCIRCRRGFLLT
SGPCKMIAPILDEIADEYQGKLTVAKLNIDQNP GTAPKYGIRGIPTLLL FKNGEVAATKVGALSKGQLKEFLDANLA

OST262 (SEQ ID NO: 207)

METDTLLLWVLLLWVPGSTGDGSM SDKIIHLTDDSFDTDLVKADGAILVDFWAEWCGPNSGGGGMIWEAWSYACGT
SGPCKMIAPILDEIADEYQGKLTVAKLNIDQNP GTAPKYGIRGIPTLLL FKNGEVAATKVGALSKGQLKEFLDANLA

OST263 (SEQ ID NO: 208)

METDTLLLWVLLLWVPGSTGDGSM SDKIIHLTDDSFDTDLVKADGAILVDFWAEWCGPNSLWIGPGDQGLFRRFVFT
SGPCKMIAPILDEIADEYQGKLTVAKLNIDQNP GTAPKYGIRGIPTLLL FKNGEVAATKVGALSKGQLKEFLDANLA

OST264 (SEQ ID NO: 209)

METDTLLLWVLLLWVPGSTGDGSVSSDQNHFRGEIEETITESFGNDHSTLDGYSRRTTLSSKMYHTKGQEGSVCLRS
SDCASGLCCARHFW SKICKPVLKEGQVCTK HRRKGSHGLEIFQRCYCGEGLS CRIQKDEHQASNSSRLHTCQRH

OST265 (SEQ ID NO: 210)

METDTLLLWVLLLWVPGSTGDGSCASPTRGGDAGVQICLACRKRKRRCMRHAMCCPGNYCKNGICVSSDQNHFRGEI
EETITESFGNDHSTLDGYSRRTTLSSKMYHTKGQEGSVCLRSSDCASGLCCARHFW SKICKPVLKEGQVCTK HRRKG
SHGLEI
FQRCYCGEGLSC.

OST266 (SEQ ID NO: 211)

MGDKIIHLTDDSFDTDLVKADGAILVDFWAEWCGPNSYAWLFSCSRCRWWLPWTSGPCKMIAPILDEIADEYQGKLT
VAKLNIDQNP GTAPKYGIRGIPTLLL FKNGEVAATKVGALSKGQLKEFLDANLA

OST267 (SEQ ID NO: 212)

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VAKLNIDQNP GTAPKYGIRGIPTLLL FKNGEVAATKVGALSKGQLKEFLDANLA

OST268 (SEQ ID NO: 213)

MGDKIIHLTDDSFDTDLVKADGAILVDFWAEWCGPNSGCTSAVCGAWAEAGREFYCTSGPCKMIAPILDEIADEYQGK
LTVAKLNIDQNP GTAPKYGIRGIPTLLL FKNGEVAATKVGALSKGQLKEFLDANLA

OST269 (SEQ ID NO: 214)

MGDKIIHLTDDSFDTDLVKADGAILVDFWAEWCGPNSLWIGPGDQGLFRRFVFTSGPCKMIAPILDEIADEYQGKLT
VAKLNIDQNP GTAPKYGIRGIPTLLL FKNGEVAATKVGALSKGQLKEFLDANLA

FIGURE 13

Effect of Dkks on Wnt1 signaling
with Coreceptors LRP5, HBM or LRP6
HOB03CE6 Cells

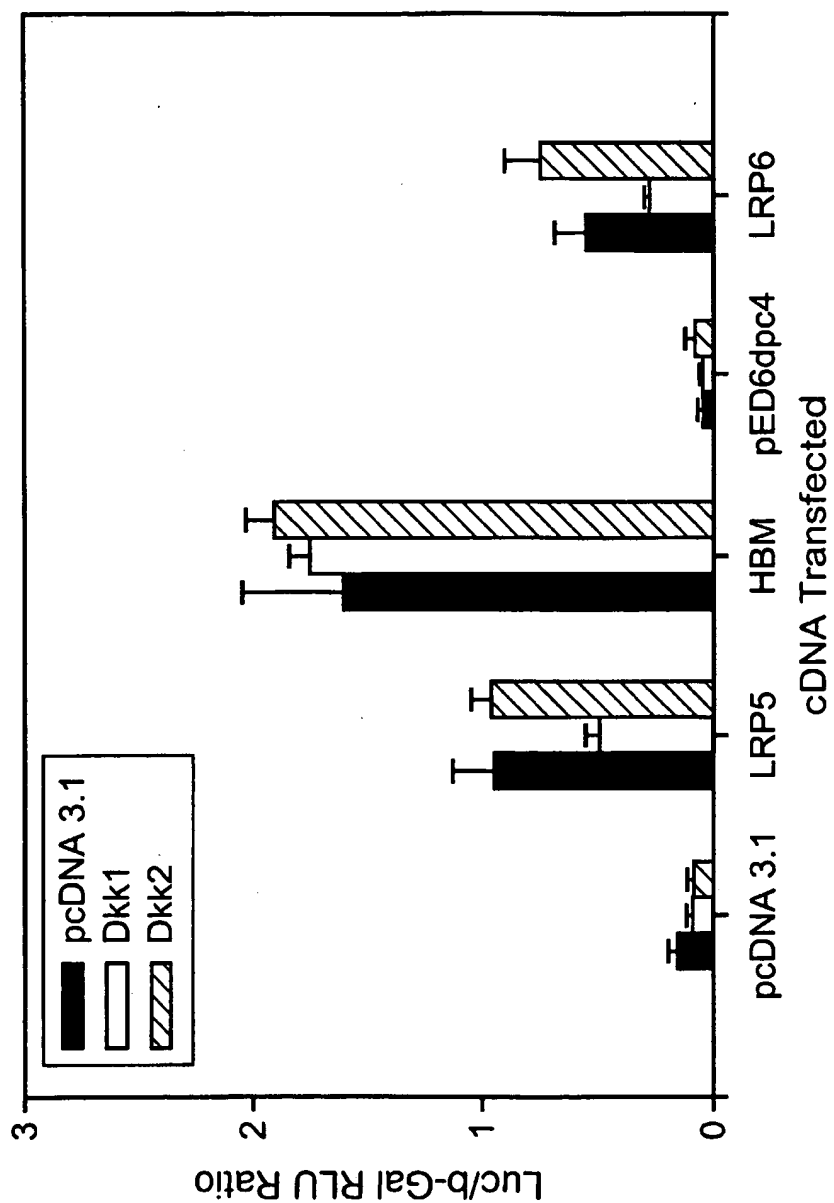


FIG. 14

Effect of Dkks on Wnt3a Signaling
with Coreceptors LRP5, HBM or LRP6
HOB03CE6 Cells

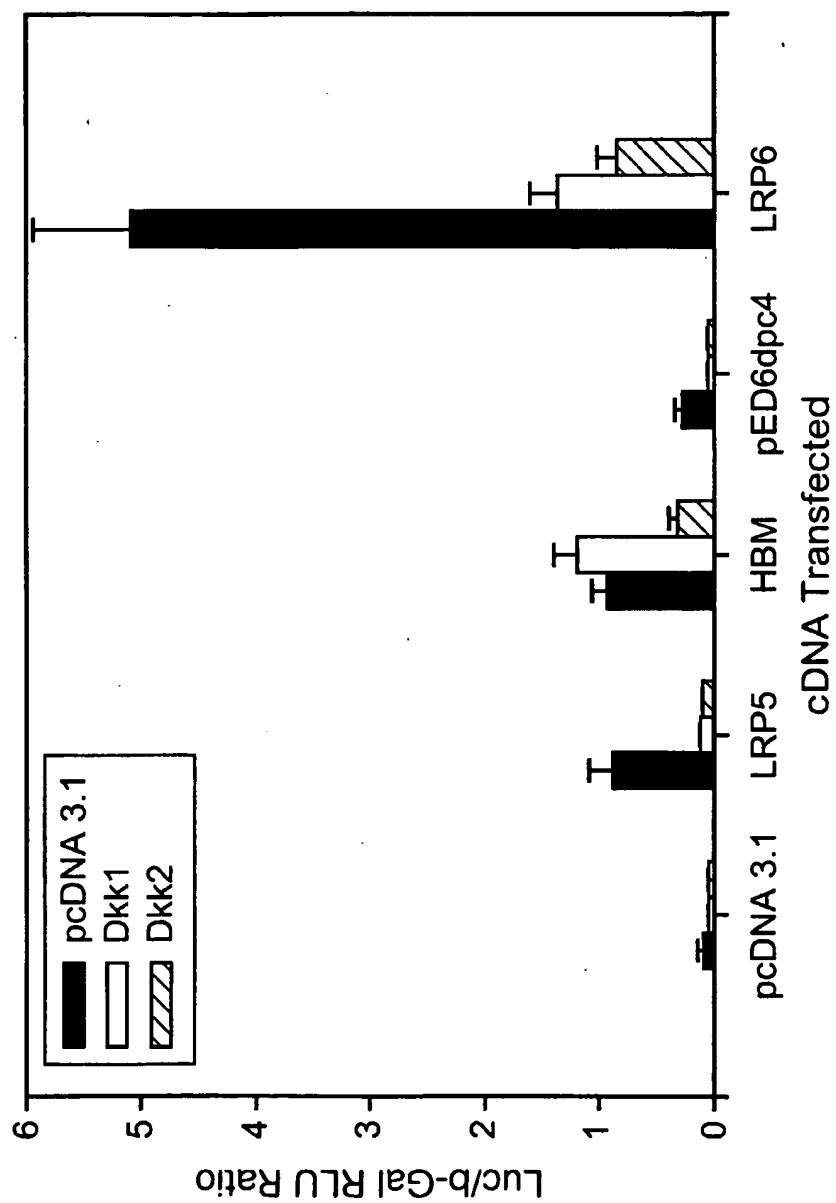


FIG. 15

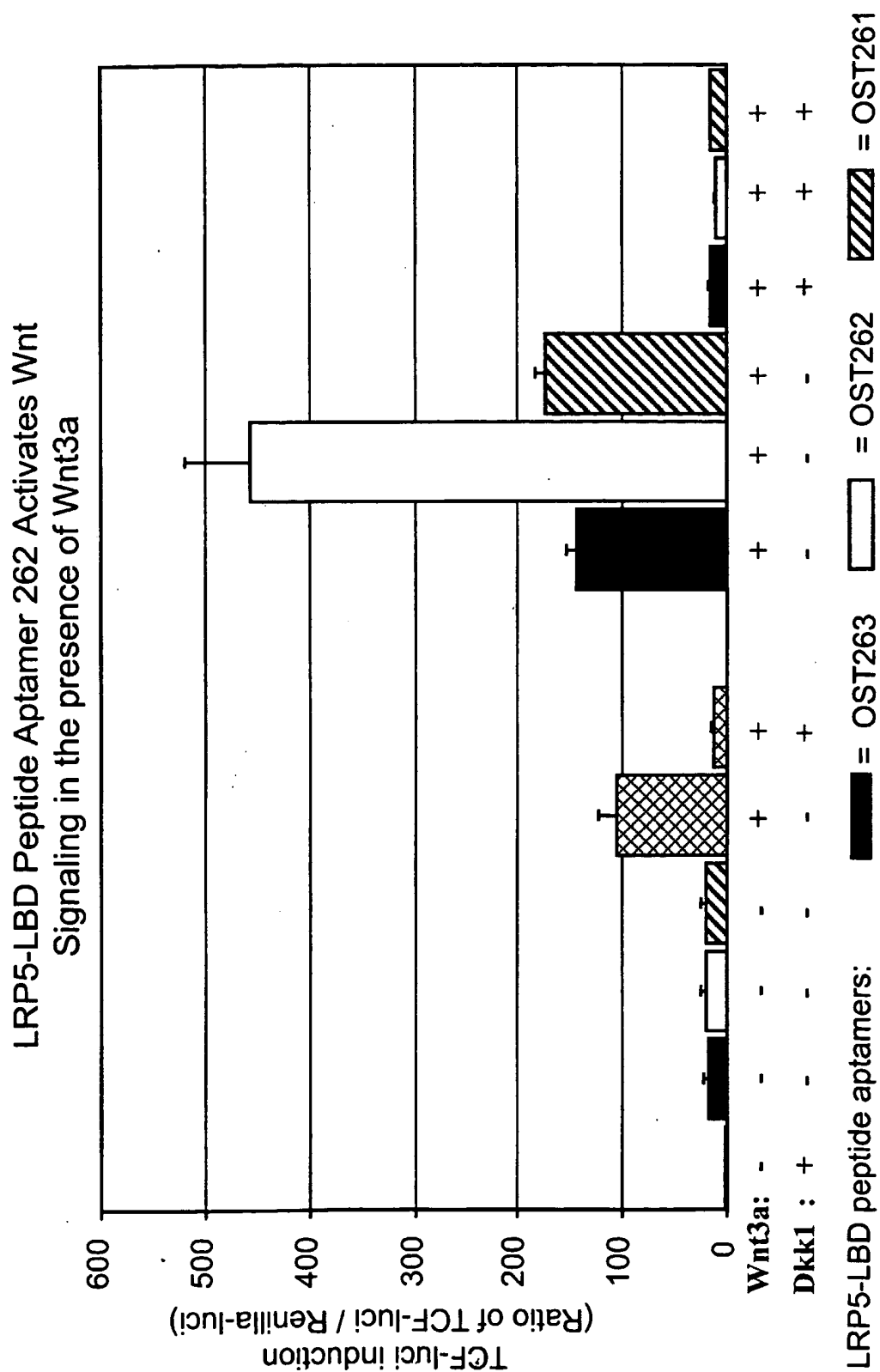
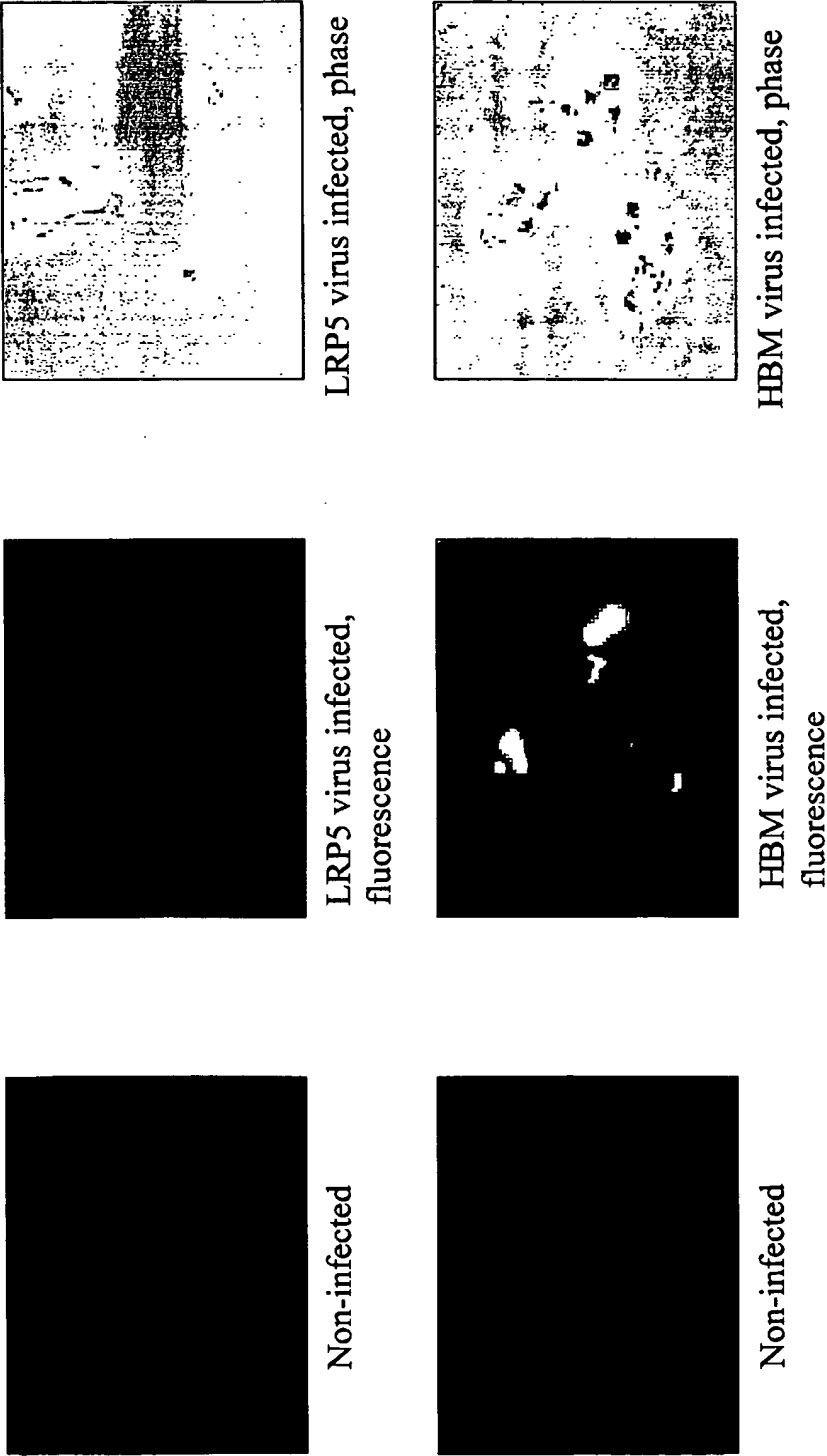
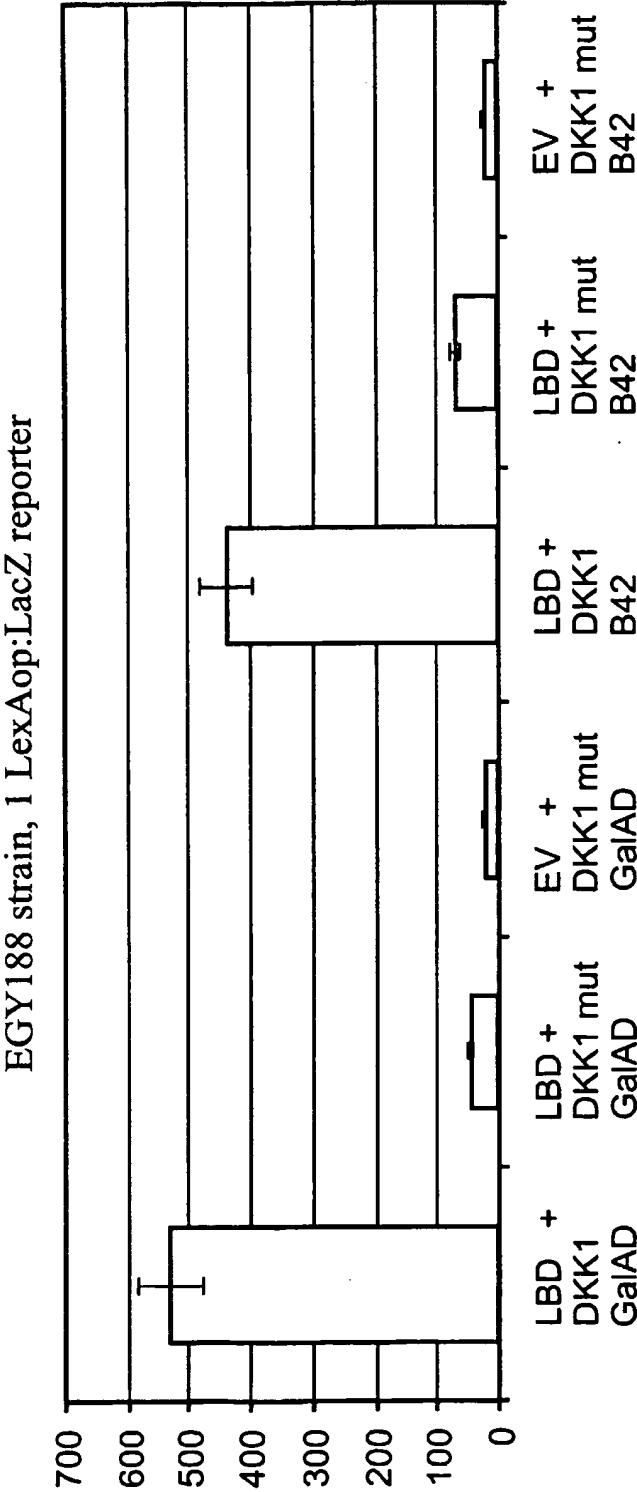


FIG. 17
Affinity Purified 69546/47



Antibody to: aa 165-177 (Mutation)



- A mutant DKK1, C220A, unable to bind to LRP5, was cloned in GalAD and B42 and tested for its ability to bind to LBD in Y2H
- Interaction LBD-DKK1 20 fold above background
- Interaction LBD-DKK1 C220A 2 to 3 fold above background
- Interaction LBD-DKK1 10 fold above LBD-DKK1 C220A mutant

FIG. 18

FIG. 19

Dkk1 Represses Wnt3a-Mediated Wnt Signaling in U2-OS Bone Cells

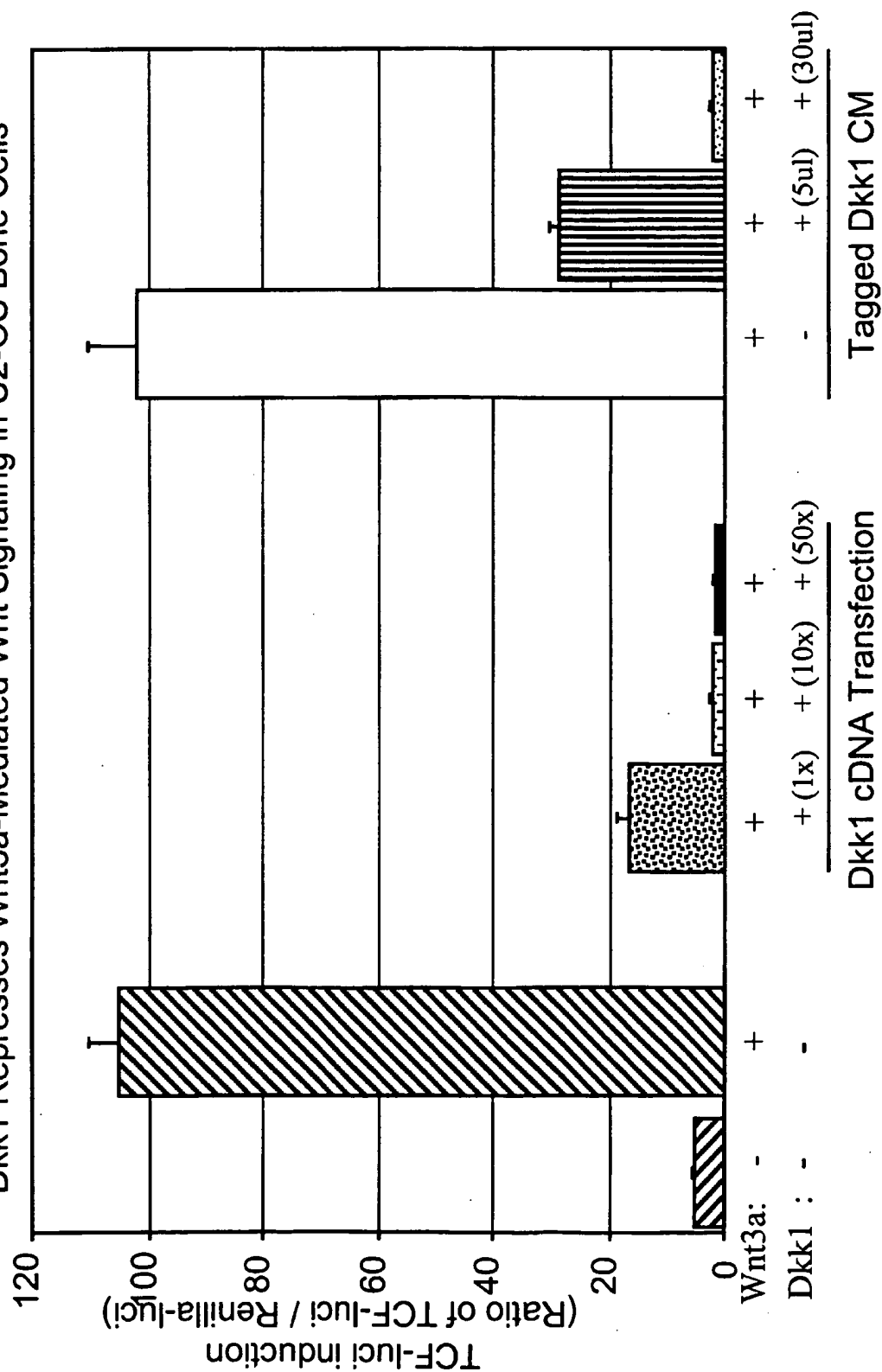
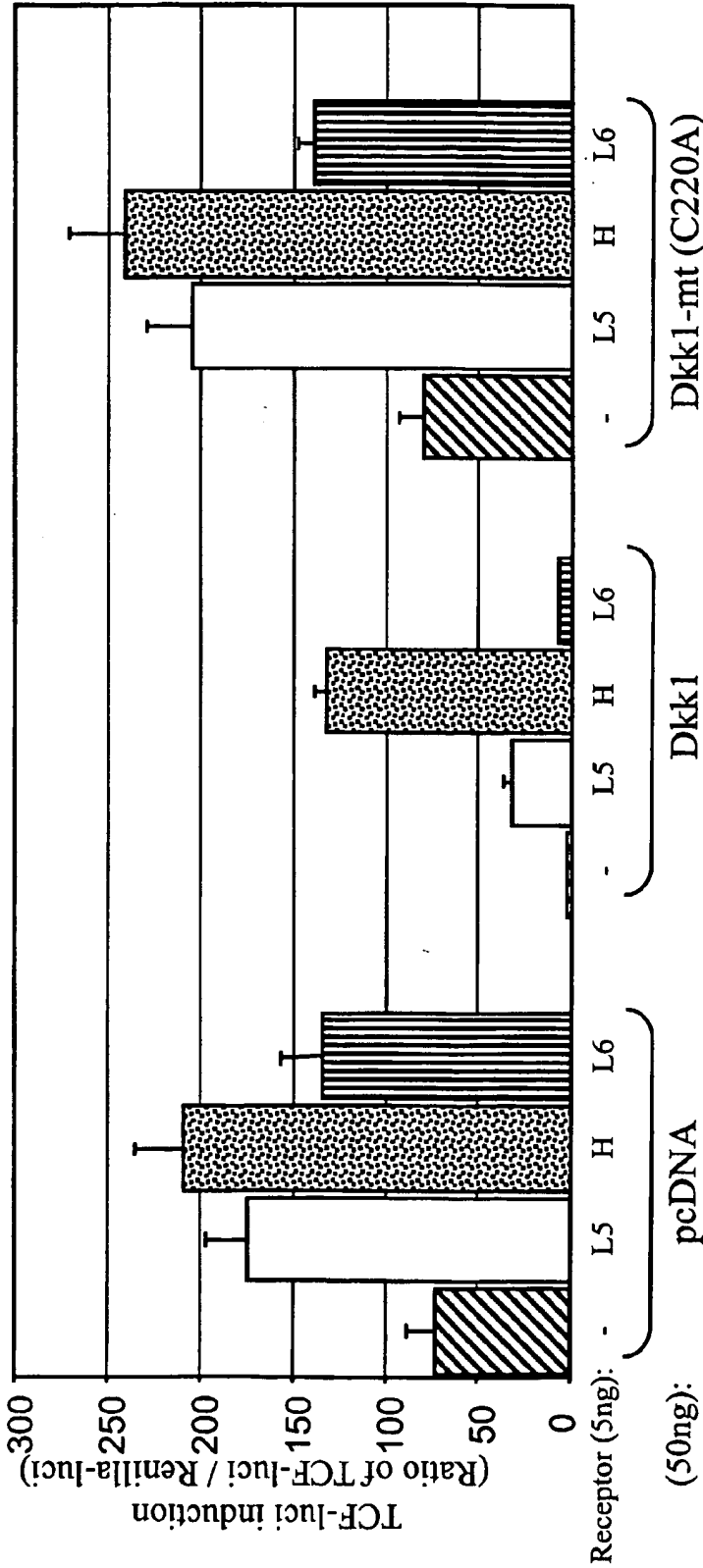


FIG. 20

Wnt1 - HBM generated TCF-luci is not efficiently inhibited by Dkk1 in U2-OS bone cells.



- With Wnt1 the TCF-signal generated by LRP5 is greater than that of LRP6.
- LRP5/6 - Wnt1 induced TCF- is efficiently blocked by Dkk1

In U2-OS cells TCF-signal can be modulated by Dkk1, Dkk1-AP,
without Wnt DNA transfection.

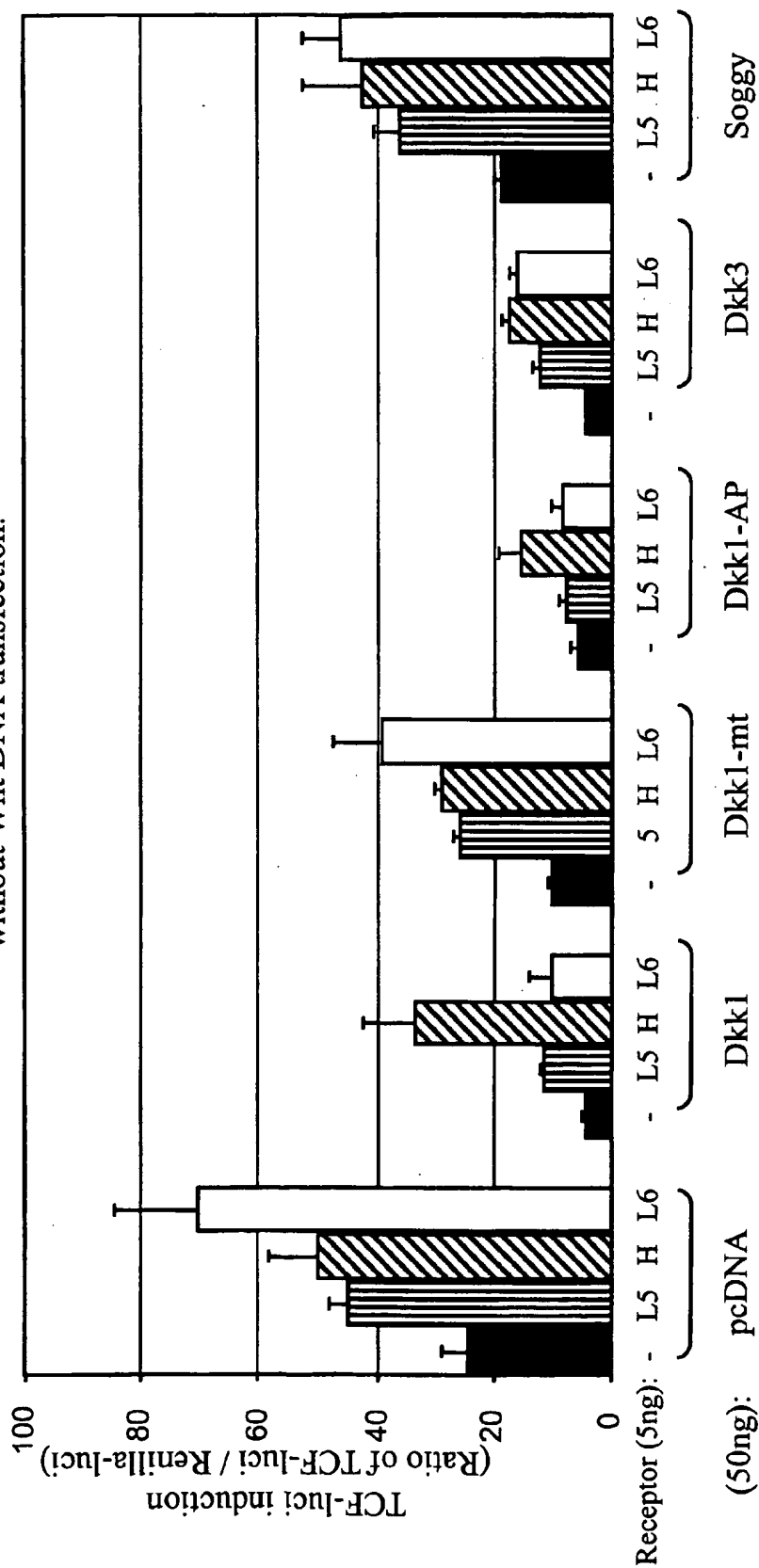


FIG. 21

FIG. 22

Aptamers 261 and 262 from the LRP5-LBD Activate Wnt Signaling in *Xenopus*



261 - LBD-Binding Peptide



262 - LBD-Binding Peptide



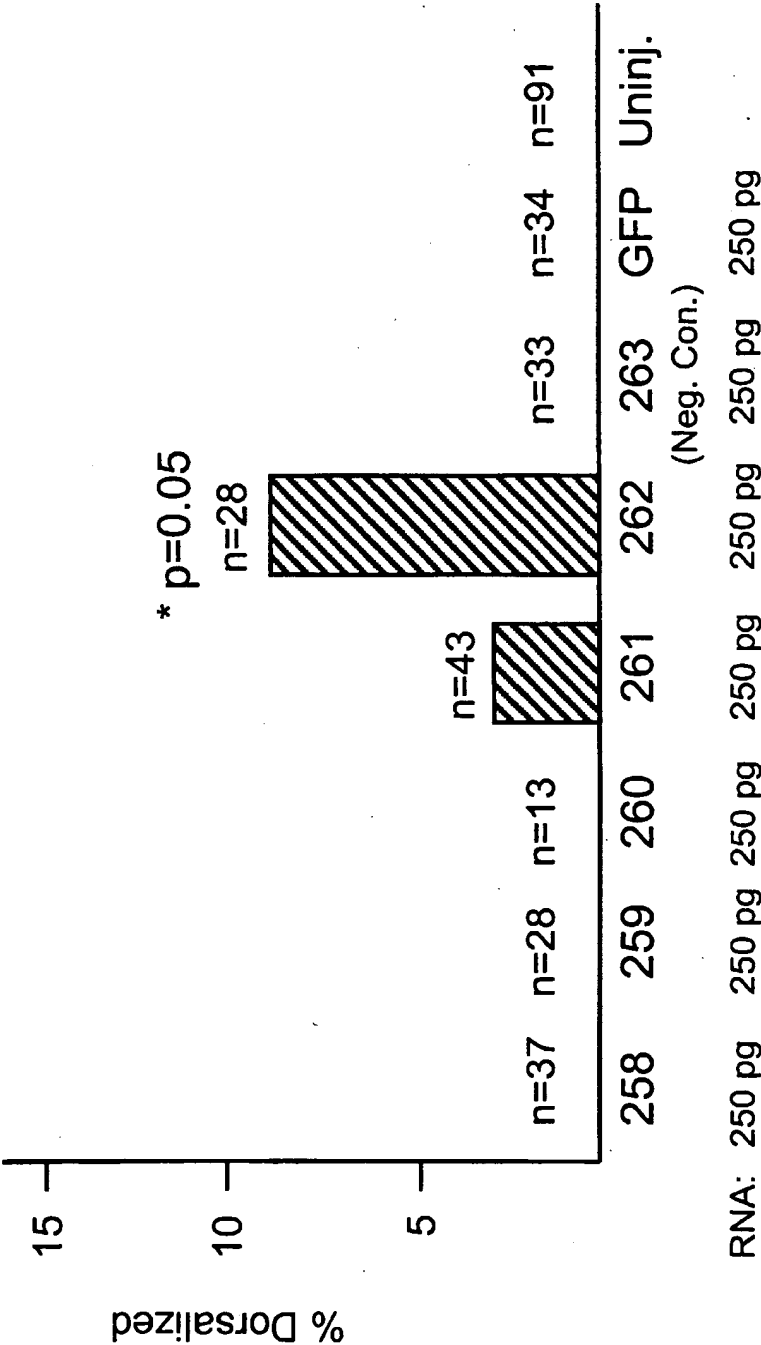
263 - Negative Control

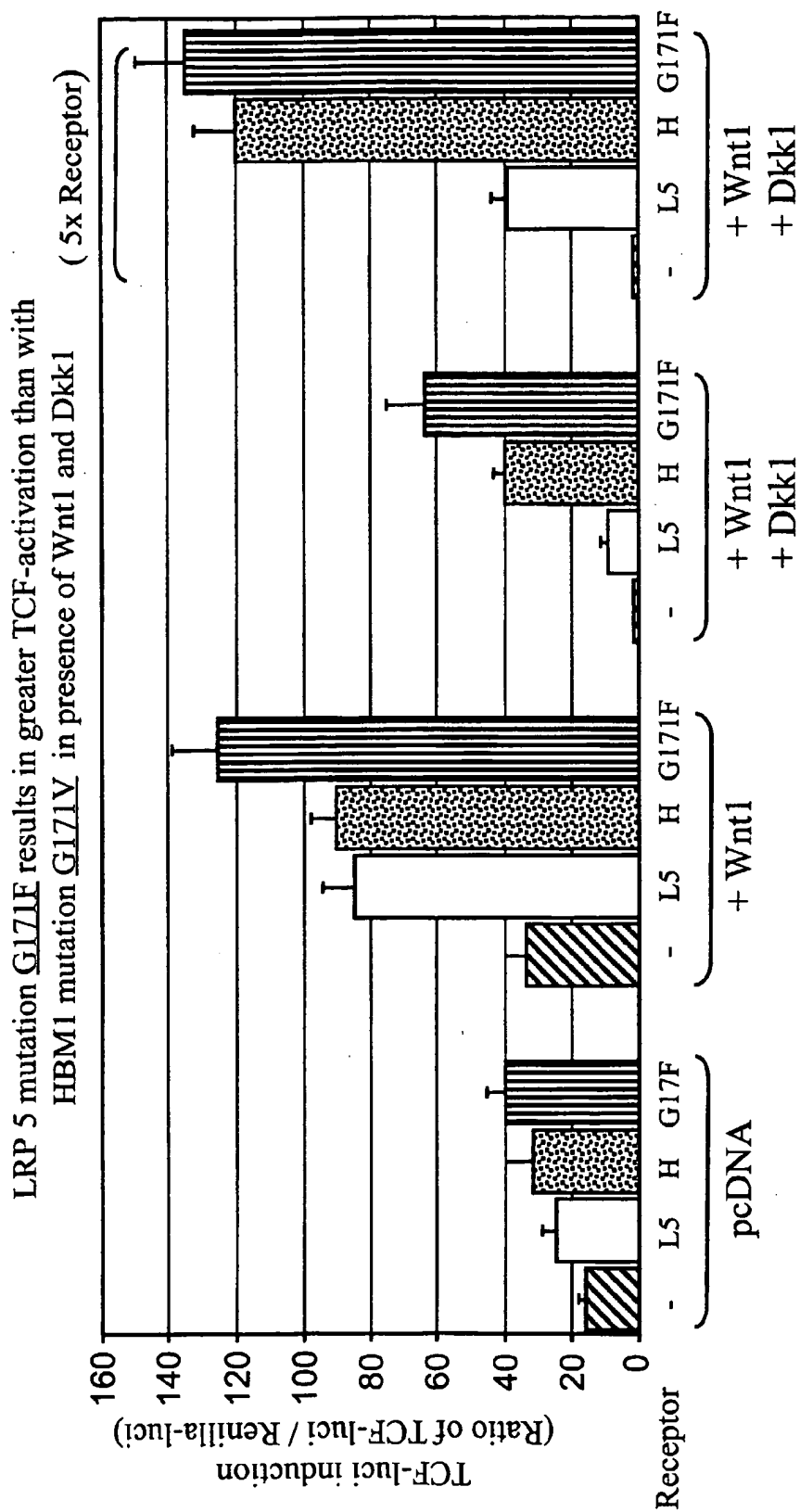


262 - LBD-Binding Peptide

FIG. 23

LRP5 Peptide Aptamers 261 and 262
Induce Wnt Signaling

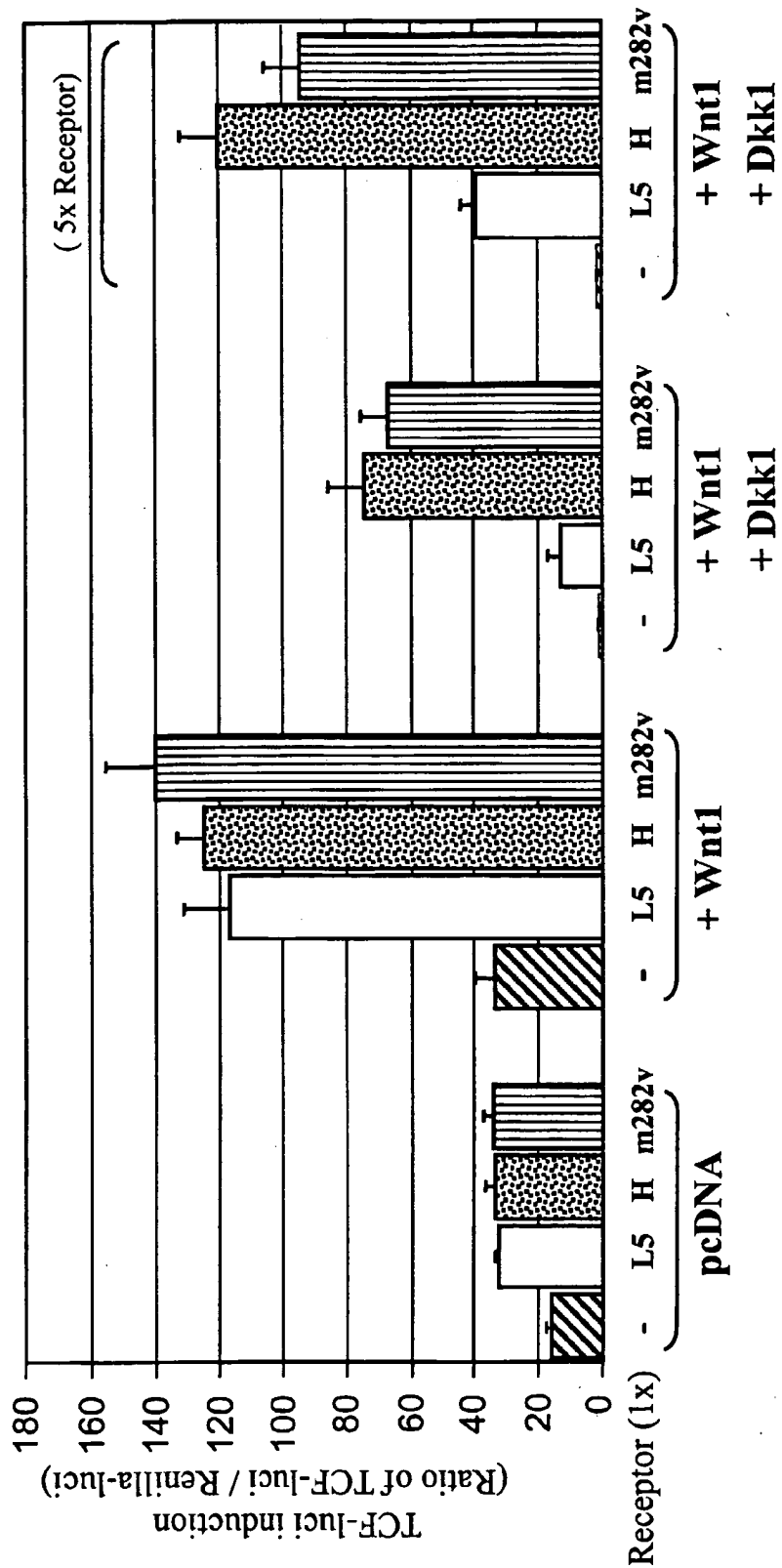




- G171F mutation involves the ringed R group (F) alteration and leads to marginally greater TCF-luciferase activation than that with HBM1 mutation G171V.

FIG. 24

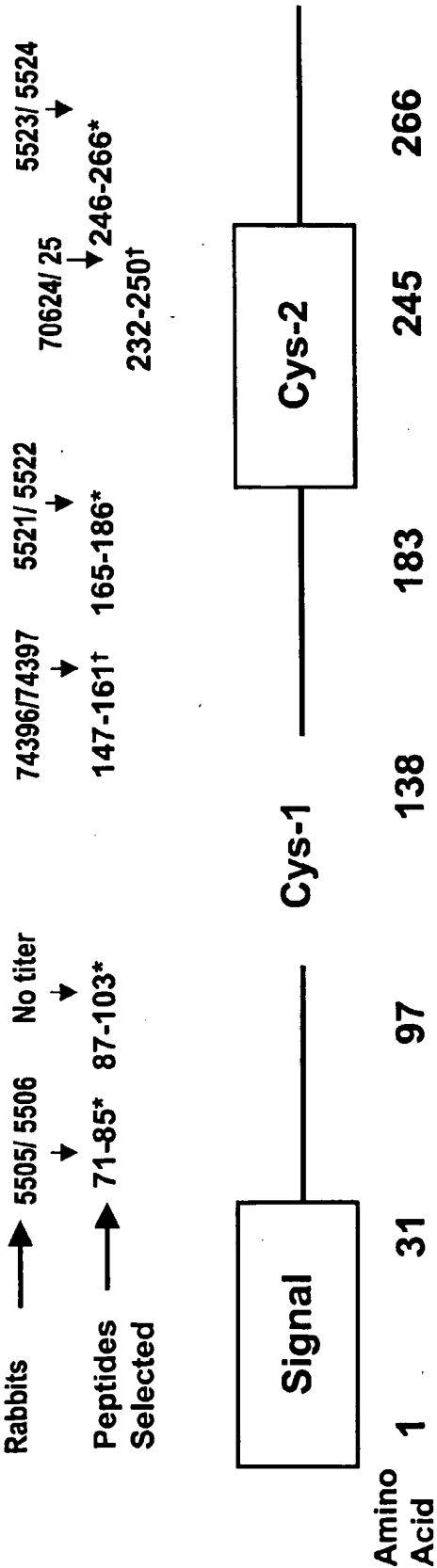
LRP5-blade1 Mutant M282V leads to HBM1 type TCF-signal
with Wnt1 and Dkk1 in U2-OS cells



- In blade 1, propeller 1, M282 is at the accessible interior position.
- It is conserved in propellers 1-3

FIG. 25

DKK1 Protein
Polyclonal Antibodies



* Sigma/Genosys
† ResGen

FIG. 26

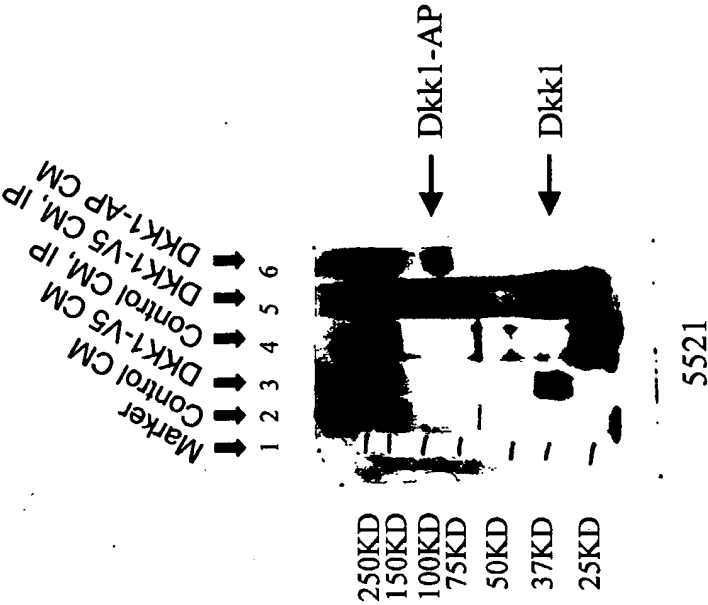


FIG. 27B

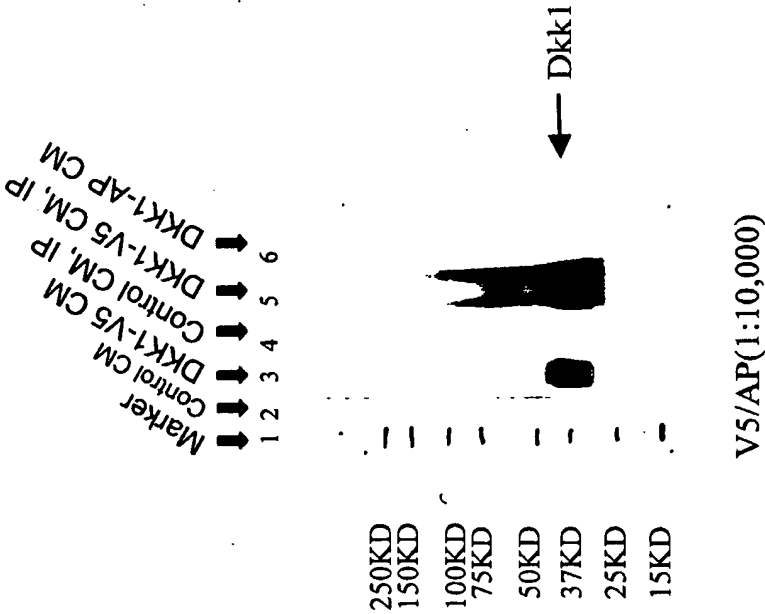


FIG. 27A

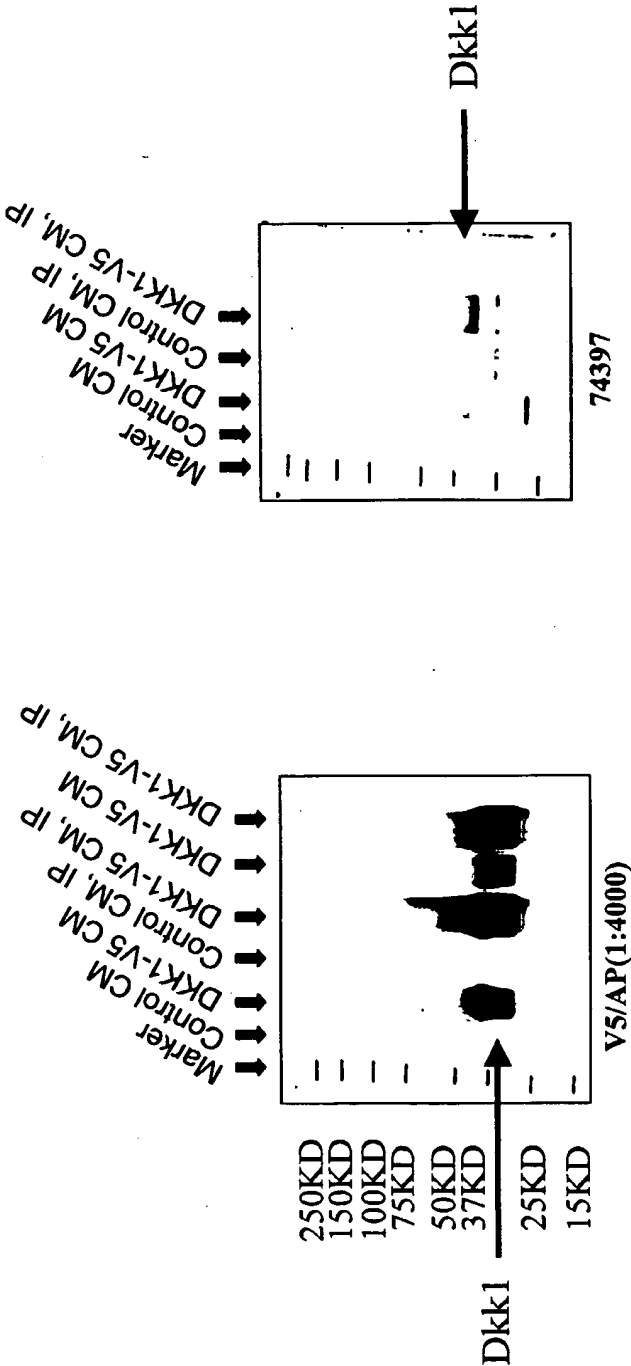


FIG. 28

032796-132.ST25

SEQUENCE LISTING

<110> Allen, Kristina M.
 Anisowicz, Anthony
 Bhat, Bheem
 Damagnez, Veronique
 Robinson, John
 Yaworsky, Paul

<120> Reagents and Method for Modulating DKK-Mediated Interactions

<130> 032796-132

<150> US 60/291,311

<151> 2001-05-17

<150> US 60/353,058

<151> 2002-02-01

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<160> 214

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<212> DNA

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           Met Glu Ala Ala Pro Pro Gly Pro Pro Trp Pro Leu Leu
           1           5           10
ctg ctg ctg ctg ctg ctg ctg gcg ctg tgc ggc tgc ccg gcc ccc gcc      157
Leu Leu Leu Leu Leu Leu Leu Ala Leu Cys Gly Cys Pro Ala Pro Ala
15           20           25
gcg gcc tcg ccg ctc ctg cta ttt gcc aac cgc cgg gac gta cgg ctg      205
Ala Ala Ser Pro Leu Leu Leu Phe Ala Asn Arg Arg Asp Val Arg Leu
30           35           40           45
gtg gac gcc ggc gga gtc aag ctg gag tcc acc atc gtg gtc agc ggc      253
Val Asp Ala Gly Gly Val Lys Leu Glu Ser Thr Ile Val Val Ser Gly
           50           55           60
ctg gag gat gcg gcc gca gtg gac ttc cag ttt tcc aag gga gcc gtg      301
Leu Glu Asp Ala Ala Val Asp Phe Gln Phe Ser Lys Gly Ala Val
65           70           75
tac tgg aca gac gtg agc gag gag gcc atc aag cag acc tac ctg aac      349
Tyr Trp Thr Asp Val Ser Glu Glu Ala Ile Lys Gln Thr Tyr Leu Asn
80           85           90
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Pro Asp Gly Leu Ala Cys Asp Trp Val Gly Lys Lys Leu Tyr Trp Thr	
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Asp Ser Glu Thr Asn Arg Ile Glu Val Ala Asn Leu Asn Gly Thr Ser	
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Arg Lys Val Leu Phe Trp Gln Asp Leu Asp Gln Pro Arg Ala Ile Ala	
145 150 155	
ttg gac ccc gct cac ggg tac atg tac tgg aca gac tgg ggt gag acg	589
Leu Asp Pro Ala His Gly Tyr Met Tyr Trp Thr Asp Trp Gly Glu Thr	
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Pro Arg Ile Glu Arg Ala Gly Met Asp Gly Ser Thr Arg Lys Ile Ile	
175 180 185	
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Thr His Pro Phe Ala Leu Thr Leu Ser Gly Asp Thr Leu Tyr Trp Thr	
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Lys Arg Lys Glu Ile Leu Ser Ala Leu Tyr Ser Pro Met Asp Ile Gln	
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Leu Tyr Trp Thr Asp Thr Gly Thr Asp Arg Ile Glu Val Thr Arg Leu	
430 435 440 445	
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Asn Gly Thr Ser Arg Lys Ile Leu Val Ser Glu Asp Leu Asp Glu Pro	
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Trp Gly Glu Asn Pro Lys Ile Glu Cys Ala Asn Leu Asp Gly Gln Glu	
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Glu Asp Lys Leu Pro His Ile Phe Gly Phe Thr Leu Leu Gly Asp Phe	
545 550 555	
atc tac tgg act gac tgg cag cgc cgc agc atc gag cgg gtg cac aag	1789
Ile Tyr Trp Thr Asp Trp Gln Arg Ser Ile Glu Arg Val His Lys	
560 565 570	
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Cys Gly Cys Ala Ser His Tyr Thr Leu Asp Pro Ser Ser Arg Asn Cys	
930 935 940	
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Ser Pro Pro Thr Thr Phe Leu Leu Phe Ser Gln Lys Ser Ala Ile Ser	
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Arg Met Ile Pro Asp Asp Gln His Ser Pro Asp Leu Ile Leu Pro Leu	
960 965 970	
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His Gly Leu Arg Asn Val Lys Ala Ile Asp Tyr Asp Pro Leu Asp Lys	
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032796-132.ST25

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His Leu Tyr Trp Ile Asp Arg Gln Gln Gln Met Ile Glu Arg Val Glu	
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Trp Arg Cys Asp Gly Phe Pro Glu Cys Asp Asp Gln Ser Asp Glu Glu	
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Gly Cys Pro Val Cys Ser Ala Ala Gln Phe Pro Cys Ala Arg Gly Gln	
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032796-132.ST25

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Val Pro Leu Tyr Asp Arg Asn His Val Thr Gly Ala Ser Ser Ser Ser	
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Pro Ser Pro Ala Thr Asp Pro Ser Leu Tyr Asn Met Asp Met Phe Tyr	
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Leu Asn Ser Asp Ser Asp Pro Tyr Pro Pro Pro Pro Thr Pro His Ser	
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Gln Tyr Leu Ser Ala Glu Asp Ser Cys Pro Pro Ser Pro Ala Thr Glu	
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032796-132.ST25

Ser Ser

1615

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 Met Glu Ala Ala Pro Pro Gly Pro Pro Trp Pro Leu Leu
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 Leu Leu Leu Leu Leu Leu Leu Ala Leu Cys Gly Cys Pro Ala Pro Ala
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 Ala Ala Ser Pro Leu Leu Leu Phe Ala Asn Arg Arg Asp Val Arg Leu
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 Val Asp Ala Gly Gly Val Lys Leu Glu Ser Thr Ile Val Val Ser Gly
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 Tyr Trp Thr Asp Val Ser Glu Glu Ala Ile Lys Gln Thr Tyr Leu Asn
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 Gln Thr Gly Ala Ala Val Gln Asn Val Val Ile Ser Gly Leu Val Ser
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 Pro Asp Gly Leu Ala Cys Asp Trp Val Gly Lys Lys Leu Tyr Trp Thr
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 Asp Ser Glu Thr Asn Arg Ile Glu Val Ala Asn Leu Asn Gly Thr Ser
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 Arg Lys Val Leu Phe Trp Gln Asp Leu Asp Gln Pro Arg Ala Ile Ala
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 Val Asp Ser Asp Ile Tyr Trp Pro Asn Gly Leu Thr Ile Asp Leu Glu
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 gag cag aag ctc tac tgg gct gac gcc aag ctc agc ttc atc cac cgt 733
 Glu Gln Lys Leu Tyr Trp Ala Asp Ala Lys Leu Ser Phe Ile His Arg

032796-132.ST25

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Thr His Pro Phe	Ala Leu Thr	Leu Ser Gly Asp	Thr Leu Tyr Trp	Thr			
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Asp Trp Gln Thr	Arg Ser Ile His	Ala Cys Asn Lys	Arg Thr Gly Gly				
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Lys Arg Lys Glu	Ile Leu Ser Ala	Leu Tyr Ser Pro	Met Asp Ile Gln				
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Val Leu Ser Gln	Glu Arg Gln Pro	Phe Phe His Thr	Arg Cys Glu Glu				
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Asp Asn Gly Gly	Cys Ser His Leu	Cys Leu Leu Ser	Pro Ser Glu Pro				
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Phe Tyr Thr Cys	Ala Cys Pro Thr	Gly Val Gln Leu	Gln Asp Asn Gly				
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Arg Thr Cys Lys	Ala Gly Ala Glu	Glu Glu Val Leu	Leu Leu Ala Arg	Arg			
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Thr Asp Leu Arg	Arg Ile Ser Leu	Asp Thr Pro Asp	Phe Thr Asp Ile				
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Val Leu Gln Val	Asp Ile Arg His	Ala Ile Ala Ile	Asp Tyr Asp				
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Pro Leu Glu Gly	Tyr Val Tyr Trp	Thr Asp Asp Glu	Val Arg Ala Ile				
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Arg Arg Ala Tyr	Leu Asp Gly Ser	Gly Ala Gln Thr	Leu Val Asn Thr				
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Glu Ile Asn Asp	Pro Asp Gly Ile	Ala Val Asp Trp	Val Ala Arg Asn				
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Leu Tyr Trp Thr	Asp Thr Gly Thr	Asp Arg Ile Glu	Val Thr Arg Leu				
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Asn Gly Thr Ser	Arg Lys Ile Leu	Val Ser Glu Asp	Leu Asp Glu Pro				
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Arg Ala Ile Ala	Leu His Pro Val	Met Gly Leu Met	Tyr Trp Thr Asp				
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Trp Gly Glu Asn	Pro Lys Ile Glu	Cys Ala Asn Leu	Asp Gly Gln Glu				
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Arg Arg Val Leu	Val Asn Ala Ser	Leu Gly Trp Pro	Asn Gly Leu Ala				
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032796-132.ST25

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Lys Ile Glu Val Ile Asn Val Asp Gly Thr Lys Arg Arg Thr Leu Leu	
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Glu Asp Lys Leu Pro His Ile Phe Gly Phe Thr Leu Leu Gly Asp Phe	
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Ile Tyr Trp Thr Asp Trp Gln Arg Arg Ser Ile Glu Arg Val His Lys	
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Val Lys Ala Ser Arg Asp Val Ile Ile Asp Gln Leu Pro Asp Leu Met	
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Gly Leu Lys Ala Val Asn Val Ala Lys Val Val Gly Thr Asn Pro Cys	
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Ala Asp Arg Asn Gly Gly Cys Ser His Leu Cys Phe Phe Thr Pro His	
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Ala Thr Arg Cys Gly Cys Pro Ile Gly Leu Glu Leu Leu Ser Asp Met	
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Lys Thr Cys Ile Val Pro Glu Ala Phe Leu Val Phe Thr Ser Arg Ala	
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Ala Ile His Arg Ile Ser Leu Glu Thr Asn Asn Asn Asp Val Ala Ile	
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ccg ctc acg ggc gtc aag gag gcc tca gcc ctg gac ttt gat gtg tcc	2125
Pro Leu Thr Gly Val Lys Glu Ala Ser Ala Leu Asp Phe Asp Val Ser	
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Asn Asn His Ile Tyr Trp Thr Asp Val Ser Leu Lys Thr Ile Ser Arg	
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Ala Phe Met Asn Gly Ser Ser Val Glu His Val Val Glu Phe Gly Leu	
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Asp Tyr Pro Glu Gly Met Ala Val Asp Trp Met Gly Lys Asn Leu Tyr	
720 725 730	
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Trp Ala Asp Thr Gly Thr Asn Arg Ile Glu Val Ala Arg Leu Asp Gly	
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Gln Phe Arg Gln Val Leu Val Trp Arg Asp Leu Asp Asn Pro Arg Ser	
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Leu Ala Leu Asp Pro Thr Lys Gly Tyr Ile Tyr Trp Thr Glu Trp Gly	
770 775 780	
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Gly Lys Pro Arg Ile Val Arg Ala Phe Met Asp Gly Thr Asn Cys Met	
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Thr Leu Val Asp Lys Val Gly Arg Ala Asn Asp Leu Thr Ile Asp Tyr	
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032796-132.ST25

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Ser	Ser	Asn	Met	Leu	Gly	Gln	Glu	Arg	Val	Val	Ile	Ala	Asp	Asp	Leu		
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Pro	His	Pro	Phe	Gly	Leu	Thr	Gln	Tyr	Ser	Asp	Tyr	Ile	Tyr	Trp	Thr		
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Arg	Ala	Ile	Val	Val	Asn	Ala	Glu	Arg	Gly	Tyr	Leu	Tyr	Phe	Thr	Asn		
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032796-132.ST25

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Glu Asp Ala Asn Ile Val Gln Pro Leu Gly Leu Thr Ile Leu Gly Lys	
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His Leu Tyr Trp Ile Asp Arg Gln Gln Gln Met Ile Glu Arg Val Glu	
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Leu Thr Gly Ile His Ala Val Glu Glu Val Ser Leu Glu Glu Phe Ser	
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gcc cac cca tgt gcc cgt gac aat ggt ggc tgc tcc cac atc tgt att	3757
Ala His Pro Cys Ala Arg Asp Asn Gly Gly Cys Ser His Ile Cys Ile	
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Ala Lys Gly Asp Gly Thr Pro Arg Cys Ser Cys Pro Val His Leu Val	
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ctc ctg cag aac ctg ctg acc tgt gga gag ccg ccc acc tgc tcc ccg	3853
Leu Leu Gln Asn Leu Leu Thr Cys Gly Glu Pro Pro Thr Cys Ser Pro	
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Asp Gln Phe Ala Cys Ala Thr Gly Glu Ile Asp Cys Ile Pro Gly Ala	
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Trp Arg Cys Asp Gly Phe Pro Glu Cys Asp Asp Gln Ser Asp Glu Glu	
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ggc tgc ccc gtg tgc tcc gcc gcc cag ttc ccc tgc gcg cgg ggt cag	3997
Gly Cys Pro Val Cys Ser Ala Ala Gln Phe Pro Cys Ala Arg Gly Gln	
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Cys Val Asp Leu Arg Leu Arg Cys Asp Gly Glu Ala Asp Cys Gln Asp	
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Arg Ser Asp Glu Val Asp Cys Asp Ala Ile Cys Leu Pro Asn Gln Phe	
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Arg Cys Ala Ser Gly Gln Cys Val Leu Ile Lys Gln Gln Cys Asp Ser	
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Phe Pro Asp Cys Ile Asp Gly Ser Asp Glu Leu Met Cys Glu Ile Thr	
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Lys Pro Pro Ser Asp Asp Ser Pro Ala His Ser Ser Ala Ile Gly Pro	
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Val Ile Gly Ile Ile Leu Ser Leu Phe Val Met Gly Gly Val Tyr Phe	
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Val Cys Gln Arg Val Val Cys Gln Arg Tyr Ala Gly Ala Asn Gly Pro	
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032796-132.ST25

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Phe Pro His Glu Tyr Val Ser Gly Thr Pro His Val Pro Leu Asn Phe
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Ile Ala Pro Gly Gly Ser Gln His Gly Pro Phe Thr Gly Ile Ala Cys
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gga aag tcc atg atg agc tcc gtg agc ctg atg ggg ggc cgg ggc ggg      4477
Gly Lys Ser Met Met Ser Ser Val Ser Leu Met Gly Gly Arg Gly Gly
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gtg ccc ctc tac gac cgg aac cac gtc aca ggg gcc tcg tcc agc agc      4525
Val Pro Leu Tyr Asp Arg Asn His Val Thr Gly Ala Ser Ser Ser Ser
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tcg tcc agc acg aag gcc acg ctg tac ccg ccg atc ctg aac ccg ccg      4573
Ser Ser Ser Thr Lys Ala Thr Leu Tyr Pro Pro Ile Leu Asn Pro Pro
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ccc tcc ccg gcc acg gac ccc tcc ctg tac aac atg gac atg ttc tac      4621
Pro Ser Pro Ala Thr Asp Pro Ser Leu Tyr Asn Met Asp Met Phe Tyr
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tct tca aac att ccg gcc act gcg aga ccg tac agg ccc tac atc att      4669
Ser Ser Asn Ile Pro Ala Thr Ala Arg Pro Tyr Arg Pro Tyr Ile Ile
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cga gga atg gcg ccc ccg acg acg ccc tgc agc acc gac gtg tgt gac      4717
Arg Gly Met Ala Pro Pro Thr Pro Cys Ser Thr Asp Val Cys Asp
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agc gac tac agc gcc agc cgc tgg aag gcc agc aag tac tac ctg gat      4765
Ser Asp Tyr Ser Ala Ser Arg Trp Lys Ala Ser Lys Tyr Tyr Leu Asp
      1550                1555                1560                1565
ttg aac tcg gac tca gac ccc tat cca ccc cca ccc acg ccc cac agc      4813
Leu Asn Ser Asp Ser Asp Pro Tyr Pro Pro Pro Pro Thr Pro His Ser
      1570                1575                1580
cag tac ctg tcg gcg gag gac agc tgc ccg ccc tcg ccc gcc acc gag      4861
Gln Tyr Leu Ser Ala Glu Asp Ser Cys Pro Pro Ser Pro Ala Thr Glu
      1585                1590                1595
agg agc tac ttc cat ctc ttc ccg ccc cct ccg tcc ccc tgc acg gac      4909
Arg Ser Tyr Phe His Leu Phe Pro Pro Pro Pro Ser Pro Cys Thr Asp
      1600                1605                1610
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Ser Ser
      1615
aaatatgaac aaagaaaaaa atatatttta tgatttataaa aataaatata attgggattt      5025
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  20                25                30
Pro Leu Leu Leu Phe Ala Asn Arg Arg Asp Val Arg Leu Val Asp Ala
  35                40                45
Gly Gly Val Lys Leu Glu Ser Thr Ile Val Val Ser Gly Leu Glu Asp

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032796-132.ST25

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Asp Val Ser Glu Glu Ala Ile Lys Gln Thr Tyr Leu Asn Gln Thr Gly		80
	85	90
Ala Ala Val Gln Asn Val Val Ile Ser Gly Leu Val Ser Pro Asp Gly		95
	100	105
Leu Ala Cys Asp Trp Val Gly Lys Lys Leu Tyr Trp Thr Asp Ser Glu		110
	115	120
Thr Asn Arg Ile Glu Val Ala Asn Leu Asn Gly Thr Ser Arg Lys Val		125
	130	135
Leu Phe Trp Gln Asp Leu Asp Gln Pro Lys Ala Ile Ala Leu Asp Pro		140
145	150	155
Ala His Gly Tyr Met Tyr Trp Thr Asp Trp Gly Glu Thr Pro Arg Ile		160
	165	170
Glu Arg Ala Gly Met Asp Gly Ser Thr Arg Lys Ile Ile Val Asp Ser		175
	180	185
Asp Ile Tyr Trp Pro Asn Gly Leu Thr Ile Asp Leu Glu Glu Gln Lys		190
	195	200
Leu Tyr Trp Ala Asp Ala Lys Leu Ser Phe Ile His Arg Ala Asn Leu		205
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Asp Gly Ser Phe Arg Gln Lys Val Val Glu Gly Ser Leu Thr His Pro		225
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Phe Ala Leu Thr Leu Ser Gly Asp Thr Leu Tyr Trp Thr Asp Trp Gln		240
	245	250
Thr Arg Ser Ile His Ala Cys Asn Lys Arg Thr Gly Gly Lys Arg Lys		255
	260	265
Glu Ile Leu Ser Ala Leu Tyr Ser Pro Met Asp Ile Gln Val Leu Ser		270
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Gln Glu Arg Gln Pro Phe Phe His Thr Arg Cys Glu Glu Asp Asn Gly		285
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Gly Trp Ser His Leu Cys Leu Leu Ser Pro Ser Glu Pro Phe Tyr Thr		300
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Cys Ala Cys Pro Thr Gly Val Gln Met Gln Asp Asn Gly Arg Thr Cys		320
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Lys Ala Gly Ala Glu Glu Val Leu Leu Leu Ala Arg Arg Thr Asp Leu		335
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Arg Arg Ile Ser Leu Asp Thr Pro Asp Phe Thr Asp Ile Val Leu Gln		350
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Val Asp Asp Ile Arg His Ala Ile Ala Ile Asp Tyr Asp Pro Leu Glu		365
	370	375
Gly Tyr Val Tyr Trp Thr Asp Asp Glu Val Arg Ala Ile Arg Arg Ala		380
385	390	395
Tyr Leu Asp Gly Ser Gly Ala Gln Thr Leu Val Asn Thr Glu Ile Asn		400
	405	410
Asp Pro Asp Gly Ile Ala Val Asp Trp Val Ala Arg Asn Leu Tyr Trp		415
	420	425
Thr Asp Thr Gly Thr Asp Arg Ile Glu Val Thr Arg Leu Asn Gly Thr		430
	435	440
Ser Arg Lys Ile Leu Val Ser Glu Asp Leu Asp Glu Pro Arg Ala Ile		445
	450	455
Ala Leu His Pro Val Met Gly Leu Met Tyr Trp Thr Asp Trp Gly Glu		460
465	470	475
Asn Pro Lys Ile Glu Cys Ala Asn Leu Asp Gly Gln Glu Arg Arg Val		480
	485	490
Leu Val Asn Ala Ser Leu Gly Trp Pro Asn Gly Leu Ala Leu Asp Leu		495
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032796-132.ST25

Gln Glu Gly Lys Leu Tyr Trp Gly Asp Ala Lys Thr Asp Lys Ile Glu
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 Val Ile Asn Val Asp Gly Thr Lys Arg Arg Thr Leu Leu Glu Asp Lys
 530 535 540
 Leu Pro His Ile Phe Gly Phe Thr Leu Leu Gly Asp Phe Ile Tyr Trp
 545 550 555 560
 Thr Asp Trp Gln Arg Arg Ser Ile Glu Arg Val His Lys Val Lys Ala
 565 570 575
 Ser Arg Asp Val Ile Ile Asp Gln Leu Pro Asp Leu Met Gly Leu Lys
 580 585 590
 Ala Val Asn Val Ala Lys Val Val Gly Thr Asn Pro Cys Ala Asp Arg
 595 600 605
 Asn Gly Gly Cys Ser His Leu Cys Phe Phe Thr Pro His Ala Thr Arg
 610 615 620
 Cys Gly Cys Pro Ile Gly Leu Glu Leu Leu Ser Asp Met Lys Thr Cys
 625 630 635 640
 Ile Val Pro Glu Ala Phe Leu Val Phe Thr Ser Arg Ala Ala Ile His
 645 650 655
 Arg Ile Ser Leu Glu Thr Asn Asn Asn Asp Val Ala Ile Pro Leu Thr
 660 665 670
 Gly Val Lys Glu Ala Ser Ala Leu Asp Phe Asp Val Ser Asn Asn His
 675 680 685
 Ile Tyr Trp Thr Asp Val Ser Leu Lys Asn Ile Ser Arg Ala Phe Met
 690 695 700
 Asn Gly Ser Ser Val Glu His Val Val Glu Phe Gly Leu Asp Tyr Pro
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 Glu Gly Met Ala Val Asp Trp Met Gly Lys Asn Leu Tyr Trp Ala Asp
 725 730 735
 Thr Gly Thr Asn Arg Ile Glu Val Ala Arg Leu Asp Gly Gln Phe Arg
 740 745 750
 Gln Val Leu Val Trp Arg Asp Leu Asp Asn Pro Arg Ser Leu Ala Leu
 755 760 765
 Asp Pro Thr Lys Gly Tyr Ile Tyr Trp Thr Glu Trp Gly Gly Lys Pro
 770 775 780
 Arg Ile Val Arg Ala Phe Met Asp Gly Thr Asn Cys Met Thr Leu Val
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 Asp Lys Val Gly Arg Ala Asn Asp Leu Thr Ile Asp Tyr Ala Asp Gln
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 Arg Leu Tyr Trp Thr Asp Leu Asp Thr Asn Met Ile Glu Ser Ser Asn
 820 825 830
 Met Leu Gly Gln Glu Arg Val Val Ile Ala Asp Asp Leu Pro His Pro
 835 840 845
 Phe Gly Leu Thr Gln Tyr Ser Asp Tyr Ile Tyr Trp Thr Asp Trp Asn
 850 855 860
 Leu His Ser Ile Glu Arg Ala Asp Lys Thr Ser Gly Arg Asn Arg Thr
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 Leu Ile Gln Gly His Leu Asp Phe Val Met Asp Ile Leu Val Phe His
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 Ser Ser Arg Gln Asp Gly Leu Asn Asp Cys Met His Asn Asn Gly Gln
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 Cys Gly Gln Leu Cys Leu Ala Ile Pro Gly Gly His Arg Cys Gly Cys
 915 920 925
 Ala Ser His Tyr Thr Leu Asp Pro Ser Ser Arg Asn Cys Ser Pro Pro
 930 935 940
 Thr Thr Phe Leu Leu Phe Ser Gln Lys Ser Ala Ile Ser Arg Met Ile
 945 950 955 960
 Pro Asp Asp Gln His Ser Pro Asp Leu Ile Leu Pro Leu His Gly Leu

032796-132.ST25

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Trp	Val	Asp	Gly	Arg	Gln	Asn	Ile	Lys	Arg	Ala	Lys	Asp	Asp	Gly	Thr	Trp	Val	Asp	Gly	Arg	Gln	Asn	Ile	Lys	Arg	Ala	Lys	Asp	Asp	Gly	Thr	Trp	Val	Asp	Gly	Arg	Gln	Asn	Ile	Lys	Arg	Ala	Lys	Asp	Asp	Gly	Thr
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Thr	Cys	Glu	Ala	Thr	Asn	Thr	Ile	Asn	Val	His	Arg	Leu	Ser	Gly	Glu	Thr	Cys	Glu	Ala	Thr	Asn	Thr	Ile	Asn	Val	His	Arg	Leu	Ser	Gly	Glu	Thr	Cys	Glu	Ala	Thr	Asn	Thr	Ile	Asn	Val	His	Arg	Leu	Ser	Gly	Glu
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Arg	Ala	Ala	Lys	Ile	Glu	Arg	Ala	Ala	Leu	Asp	Gly	Thr	Glu	Arg	Glu	Arg	Ala	Ala	Lys	Ile	Glu	Arg	Ala	Ala	Leu	Asp	Gly	Thr	Glu	Arg	Glu	Arg	Ala	Ala	Lys	Ile	Glu	Arg	Ala	Ala	Leu	Asp	Gly	Thr	Glu	Arg	Glu
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032796-132.ST25

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 Tyr Asp Arg Asn His Val Thr Gly Ala Ser Ser Ser Ser Ser Ser
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 Thr Lys Ala Thr Leu Tyr Pro Pro Ile Leu Asn Pro Pro Pro Ser Pro
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 Ala Thr Asp Pro Ser Leu Tyr Asn Met Asp Met Phe Tyr Ser Ser Asn
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 Gly Gly Val Lys Leu Glu Ser Thr Ile Val Val Ser Gly Leu Glu Asp
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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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<212> DNA
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<223> Primer

<400> 31
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16

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<212> DNA
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<220>
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<400> 32
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<220>
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<400> 33
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032796-132.ST25

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<210> 36
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<213> Artificial Sequence

<220>
<223> Primer

<400> 36
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<210> 37
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 37
gccgtaatgc ggcacaggga ataagct 27

<210> 38
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 38
gagaggctat atccctgggc 20

<210> 39
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032796-132.ST25

<220>

<223> Primer

<400> 39

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20

<210> 40

<211> 163

<212> DNA

<213> Homo sapiens

<400> 40

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<211> 419

<212> DNA

<213> Homo sapiens

<400> 41

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ccggcgagg	caagctggag	tccaccatcg	tggtcagcgg	cctggaggat	gcggccgcag	120
tggaactcca	gttttccaag	ggagccgtgt	actggacaga	cgtgagcgag	gaggccatca	180
agcagaccta	cctgaaccag	acggggggccg	ccgtgcagaa	cgtggtcatc	tccggcctgg	240
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<210> 42

<211> 221

<212> DNA

<213> Homo sapiens

<400> 42

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gggatggatg	gcagcaccg	gaagatcatt	gtggactcgg	acatttactg	gccaatgga	120
ctgaccatcg	acctggagg	gcagaagctc	tactgggctg	acgccaagct	cagcttcac	180
caccgtgcc	acctggacgg	ctcgttccg	taggtacca	c		221

<210> 43

<211> 221

<212> DNA

<213> Homo sapiens

<400> 43

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agcgactgg	ggggaagagg	aaggagatcc	tgagtgcct	atactcacc	atggacatcc	180
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032796-132.ST25

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 <211> 156
 <212> DNA
 <213> Homo sapiens

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 gtccccaagc gagccttttt acacatgcgc ctgccccacg ggtgtgcaga tgcaggacaa 120
 cggcaggacg tgtaaggcag gtgaggcggt gggacg 156

<210> 45
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 <212> DNA
 <213> Homo sapiens

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 tgccatcgac tacgaccgc tagagggcta tgtctactgg acagatgacg aggtgcgggc 180
 catccgcagg gcgtacctgg acgggtctgg ggcgagacg ctggtcaaca ccgagatcaa 240
 cgaccccgat ggcatcgcg tgcactgggt ggcccgaac ctctactgga ccgacacggg 300
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<210> 46
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 <212> DNA
 <213> Homo sapiens

<400> 46
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 aacttgatg ggcaggagcg gcgtgtgctg gtcaatgcct ccctcgggtg gcccacggc 120
 ctggccctgg acctgcagga ggggaagctc tactggggag acgccaagac agacaagatc 180
 gaggtgaggc tcctgtgg 198

<210> 47
 <211> 244
 <212> DNA
 <213> Homo sapiens

<400> 47
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 tcccgacat tttcgggttc acgctgctgg gggacttcat ctactggact gactggcagc 120
 gccgcagcat cgagcgggtg cacaaggta aggccagccg ggacgtcatc attgaccagc 180
 tgcccgacct gatggggctc aaagctgtga atgtggccaa ggtcgtcggg gagtccgggg 240
 ggtc 244

<210> 48
 <211> 313
 <212> DNA
 <213> Homo sapiens

032796-132.ST25

<400> 48

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tgaagacctg	catcgtgcct	gaggcctttt	tggctctcac	cagcagagcc	gccatccaca	180
ggatctccct	cgagaccaat	aacaacgacg	tggccatccc	gtcacgggc	gtcaaggagg	240
cctcagccct	ggactttgat	gtgtccaaca	accacatcta	ctggacagac	gtcagcctga	300
aggtagcgtg	ggc					313

<210> 49

<211> 255

<212> DNA

<213> Homo sapiens

<400> 49

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gggcccacac	tgggaccaac	agaatcgaag	tggcgcggt	ggacgggag	ttccggcaag	180
tcctcgtgtg	gagggacttg	gacaaccgga	ggtcgctggc	cctggatccc	accaaggggt	240
aagtgtttgc	ctgtc					255

<210> 50

<211> 210

<212> DNA

<213> Homo sapiens

<400> 50

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accattgact	acgctgacca	gcgcctctac	tggaccgacc	tggacaccaa	catgatcgag	180
tcgtccaaca	tgctgggtga	gggcccggct				210

<210> 51

<211> 352

<212> DNA

<213> Homo sapiens

<400> 51

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ccgacaagac	tagcggccgg	aaccgcaccc	tcatccaggg	ccacctggac	ttcgtgatgg	180
acatcctggt	gttccactcc	tcccgccagg	atggcctcaa	tgactgtatg	cacaacaacg	240
ggcagtggtg	gcagctgtgc	cttgccatcc	ccggcgggca	ccgctgcggc	tgcgcctcac	300
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<210> 52

<211> 225

<212> DNA

<213> Homo sapiens

<400> 52

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aaagccatcg	actatgaccc	actggacaag	ttcatctact	gggtggatgg	gcgccagaa	180

032796-132.ST25

atcaagcgag ccaaggacga cgggacccag gcaggtgccc tgtgg 225

<210> 53
<211> 235
<212> DNA
<213> Homo sapiens

<400> 53
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caataccatc aacgtccaca ggctgagcgg ggaagccatg ggggtggtgc tgcgtgggga 180
ccgcgacaag cccagggcc a tcgtcgtcaa cgcggagcga gggtaggagg ccaac 235

<210> 54
<211> 218
<212> DNA
<213> Homo sapiens

<400> 54
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gtggccctgg tggtaggaaa cacactgggc aagctgttct gggtaggagc ggacctgaag 180
cgcatgtaga gctgtgacct gtcaggtacg cgccccgg 218

<210> 55
<211> 234
<212> DNA
<213> Homo sapiens

<400> 55
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gtgtggagaa gaccaccggg gacaagcgga ctgcaccca gggccgtgtc gcccacctca 180
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<210> 56
<211> 157
<212> DNA
<213> Homo sapiens

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ttgccaaagg tgatgggaca ccacggtgct catgcccagt ccacctcgtg ctctgcaga 120
acctgctgac ctgtggaggt aggtgtgacc taggtgc 157

<210> 57
<211> 272
<212> DNA
<213> Homo sapiens

<400> 57
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032796-132.ST25

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gggtcagtgt	gtggacctgc	gcctgcgctg	cgacggcgag	gcagactgtc	aggaccgctc	240
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	134

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	274

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 <212> DNA
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032796-132.ST25

<400> 62

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gccagcaagt	actacctgga	tttgaactcg	gactcagacc	cctatccacc	cccacccacg	180
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<212> DNA

<213> Homo sapiens

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032796-132.ST25

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032796-132.ST25

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<212> DNA

<213> Homo sapiens

<400> 66

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032796-132.ST25

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<212> DNA

<213> Homo sapiens

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032796-132.ST25

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<212> DNA

<213> Homo sapiens

<400> 68

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032796-132.ST25

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<211> 3513

<212> DNA

<213> Homo sapiens

<400> 69

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032796-132.ST25

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<212> DNA

<213> Homo sapiens

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032796-132.ST25

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<213> Homo sapiens

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032796-132.ST25

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<213> Homo sapiens

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032796-132.ST25

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<211> 1683

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<213> Homo sapiens

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032796-132.ST25

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<212> DNA

<213> Homo sapiens

<400> 74

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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<213> Homo sapiens

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<213> Homo sapiens

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032796-132.ST25

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<212> DNA

<213> Homo sapiens

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032796-132.ST25

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<211> 2306

<212> DNA

<213> Homo sapiens

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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032796-132.ST25

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caccagaatg	atgttcgacc	tatccgtagc	attaaaaact	ttctgcagag	caggcttctg	8640
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taatctgaat	tttcaagaga	aaacaagcaa	acataactga	tcagctgccg	gtatgttcat	8760
cgtgtgaaat	tgcatcccaa	gtgagctgtg	ctcagcagtg	cttgacaca	gagctgcaag	8820
ctgcgctggg	gtggaggacc	gtcacttaca	ctctgccaa	gacggaggtc	gcattgctgt	8880
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<210> 86

<211> 1204

<212> DNA

<213> Homo sapiens

<400> 86

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tggtgcagaa	gtagtaaga	atgagactgg	tggtggagaa	ggaattgaag	tcttaaagaa	180
tgaaccttat	gagaaggatg	gagaaaagg	acagtatacg	cacaaaattt	atcacctaaa	240
gagcaaaagt	cctgcattcg	tgaggatgat	tgctcccgag	ggctccttgg	tgtttcatga	300
gaaagcctgg	aatgcgtacc	cctactgtag	aacaattgta	acgaatgaat	atatgaaaga	360
tgatttcttc	attaaaatcg	aaacatggca	caaaccagac	ttgggaacat	tagaaaatgt	420

032796-132.ST25

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acatgggtta gatccaaaca catggaaaac tgttgaaatt gtccatatag atattgcaga 480
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agtcaagacc aagagaggcc ctttgggacc caactggaag aaggagctgg caaacagccc 600
tgactgtccc cagatgtgtg cctataagct ggtgaccatc aaattcaagt ggtggggact 660
gcaaagcaaa gtagaaaact tcattcaaaa gcaagaaaaa cggatattta caaacttcca 720
tcgccagctt ttttgttga ttgacaagtg gatcgatctc acgatggaag acattaggag 780
aatggaagac gagactcaga aagaactaga aacaatgcgt aagaggggtt ccgttcgagg 840
cacgtcggct gctgatgtct agatgagtc cctgtagggt cagagacaat gtcaaactgt 900
ttacgtaatc aaggtcaagt gaggggaaca agcgcagcca gtgatgagt aacaacaatc 960
tgaccagtat cttgcagtgt tgacgtttcc cagatgtgtg cttgtgatga tacacacaca 1020
tgcacagggt ctcaaccacg tgtgtatata tgtatgtgtg catatgtctg tagctgtata 1080
taaagcgcag gtagagctac agatccagat acacacactt gtgtatatat gtacatacag 1140
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<210> 87
 <211> 892
 <212> PRT
 <213> Homo sapiens

<400> 87

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Met Asp His Tyr Asp Ser Gln Gln Thr Asn Asp Tyr Met Gln Pro Glu
 1          5          10          15
Glu Asp Trp Asp Arg Asp Leu Leu Leu Asp Pro Ala Trp Glu Lys Gln
          20          25          30
Gln Arg Lys Thr Phe Thr Ala Trp Cys Asn Ser His Leu Arg Lys Ala
          35          40          45
Gly Thr Gln Ile Glu Asn Ile Glu Glu Asp Phe Arg Asp Gly Leu Lys
          50          55          60
Leu Met Leu Leu Leu Glu Val Ile Ser Gly Glu Arg Leu Ala Lys Pro
          65          70          75          80
Glu Arg Gly Lys Met Arg Val His Lys Ile Ser Asn Val Asn Lys Ala
          85          90          95
Leu Asp Phe Ile Ala Ser Lys Gly Val Lys Leu Val Ser Ile Gly Ala
          100          105          110
Glu Glu Ile Val Asp Gly Asn Val Lys Met Thr Leu Gly Met Ile Trp
          115          120          125
Thr Ile Ile Leu Arg Phe Ala Ile Gln Asp Ile Ser Val Glu Glu Thr
          130          135          140
Ser Ala Lys Glu Gly Leu Leu Leu Trp Cys Gln Arg Lys Thr Ala Pro
          145          150          155          160
Tyr Lys Asn Val Asn Ile Gln Asn Phe His Ile Ser Trp Lys Asp Gly
          165          170          175
Leu Gly Phe Cys Ala Leu Ile His Arg His Arg Pro Glu Leu Ile Asp
          180          185          190
Tyr Gly Lys Leu Arg Lys Asp Asp Pro Leu Thr Asn Leu Asn Thr Ala
          195          200          205
Phe Asp Val Ala Glu Lys Tyr Leu Asp Ile Pro Lys Met Leu Asp Ala
          210          215          220
Glu Asp Ile Val Gly Thr Ala Arg Pro Asp Glu Lys Ala Ile Met Thr
          225          230          235          240
Tyr Val Ser Ser Phe Tyr His Ala Phe Ser Gly Ala Gln Lys Ala Glu
          245          250          255
Thr Ala Ala Asn Arg Ile Cys Lys Val Leu Ala Val Asn Gln Glu Asn
          260          265          270
Glu Gln Leu Met Glu Asp Tyr Glu Lys Leu Ala Ser Asp Leu Leu Glu
          275          280          285

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032796-132.ST25

Trp Ile Arg Arg Thr Ile Pro Trp Leu Glu Asn Arg Val Pro Glu Asn
 290 295 300
 Thr Met His Ala Met Gln Lys Leu Glu Asp Phe Arg Asp Tyr Arg
 305 310 315 320
 Arg Leu His Lys Pro Pro Lys Val Gln Glu Lys Cys Gln Leu Glu Ile
 325 330 335
 Asn Phe Asn Thr Leu Gln Thr Lys Leu Arg Leu Ser Asn Arg Pro Ala
 340 345 350
 Phe Met Pro Ser Glu Gly Arg Met Val Ser Asp Ile Asn Asn Ala Trp
 355 360 365
 Gly Cys Leu Glu Gln Val Glu Lys Gly Tyr Glu Glu Trp Leu Leu Asn
 370 375 380
 Glu Ile Arg Arg Leu Glu Arg Leu Asp His Leu Ala Glu Lys Phe Arg
 385 390 395 400
 Gln Lys Ala Ser Ile His Glu Ala Trp Thr Asp Gly Lys Glu Ala Met
 405 410 415
 Leu Arg Gln Lys Asp Tyr Glu Thr Ala Thr Leu Ser Glu Ile Lys Ala
 420 425 430
 Leu Leu Lys Lys His Glu Ala Phe Glu Ser Asp Leu Ala Ala His Gln
 435 440 445
 Asp Arg Val Glu Gln Ile Ala Ala Ile Ala Gln Glu Leu Asn Glu Leu
 450 455 460
 Asp Tyr Tyr Asp Ser Pro Ser Val Asn Ala Arg Cys Gln Lys Ile Cys
 465 470 475 480
 Asp Gln Trp Asp Asn Leu Gly Ala Leu Thr Gln Lys Arg Arg Glu Ala
 485 490 495
 Leu Glu Arg Thr Glu Lys Leu Leu Glu Thr Ile Asp Gln Leu Tyr Leu
 500 505 510
 Glu Tyr Ala Lys Arg Ala Ala Pro Phe Asn Asn Trp Met Glu Gly Ala
 515 520 525
 Met Glu Asp Leu Gln Asp Thr Phe Ile Val His Thr Ile Glu Glu Ile
 530 535 540
 Gln Gly Leu Thr Thr Ala His Glu Gln Phe Lys Ala Thr Leu Pro Asp
 545 550 555 560
 Ala Asp Lys Glu Arg Leu Ala Ile Leu Gly Ile His Asn Glu Val Ser
 565 570 575
 Lys Ile Val Gln Thr Tyr His Val Asn Met Ala Gly Thr Asn Pro Tyr
 580 585 590
 Thr Thr Ile Thr Pro Gln Glu Ile Asn Gly Lys Trp Asp His Val Arg
 595 600 605
 Gln Leu Val Pro Arg Arg Asp Gln Ala Leu Thr Glu Glu His Ala Arg
 610 615 620
 Gln Gln His Asn Glu Arg Leu Arg Lys Gln Phe Gly Ala Gln Ala Asn
 625 630 635 640
 Val Ile Gly Pro Trp Ile Gln Thr Lys Met Glu Glu Ile Gly Arg Ile
 645 650 655
 Ser Ile Glu Met His Gly Thr Leu Glu Asp Gln Leu Ser His Leu Arg
 660 665 670
 Gln Tyr Glu Lys Ser Ile Val Asn Tyr Lys Pro Lys Ile Asp Gln Leu
 675 680 685
 Glu Gly Asp His Gln Leu Ile Gln Glu Ala Leu Ile Phe Asp Asn Lys
 690 695 700
 His Thr Asn Tyr Thr Met Glu His Ile Arg Val Gly Trp Glu Gln Leu
 705 710 715 720
 Leu Thr Thr Ile Ala Arg Thr Ile Asn Glu Val Glu Asn Gln Ile Leu
 725 730 735
 Thr Arg Asp Ala Lys Gly Ile Ser Gln Glu Gln Met Asn Glu Phe Arg

032796-132.ST25

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      740              745              750
Ala Ser Phe Asn His Phe Asp Arg Asp His Ser Gly Thr Leu Gly Pro
      755              760              765
Glu Glu Phe Lys Ala Cys Leu Ile Ser Leu Gly Tyr Asp Ile Gly Asn
      770              775              780
Asp Pro Gln Gly Glu Ala Glu Phe Ala Arg Ile Met Ser Ile Val Asp
      785              790              795              800
Pro Asn Arg Leu Gly Val Val Thr Phe Gln Ala Phe Ile Asp Phe Met
      805              810              815
Ser Arg Glu Thr Ala Asp Thr Asp Thr Ala Asp Gln Val Met Ala Ser
      820              825              830
Phe Lys Ile Leu Ala Gly Asp Lys Asn Tyr Ile Thr Met Asp Glu Leu
      835              840              845
Arg Arg Glu Leu Pro Pro Asp Gln Ala Glu Tyr Cys Ile Ala Arg Met
      850              855              860
Ala Pro Tyr Thr Gly Pro Asp Ser Val Pro Gly Ala Leu Asp Tyr Met
      865              870              875              880
Ser Phe Ser Thr Ala Leu Tyr Gly Glu Ser Asp Leu
      885              890

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<210> 88

<211> 197

<212> PRT

<213> Homo sapiens

<400> 88

```

Met Met Phe Pro Gln Ser Arg His Ser Gly Ser Ser His Leu Pro Gln
  1              5              10              15
Gln Leu Lys Phe Thr Thr Ser Asp Ser Cys Asp Arg Ile Lys Asp Glu
      20              25              30
Phe Gln Leu Leu Gln Ala Gln Tyr His Ser Leu Lys Leu Glu Cys Asp
      35              40              45
Lys Leu Ala Ser Glu Lys Ser Glu Met Gln Arg His Tyr Val Met Tyr
      50              55              60
Tyr Glu Met Ser Tyr Gly Leu Asn Ile Glu Met His Lys Gln Ala Glu
      65              70              75              80
Ile Val Lys Arg Leu Asn Gly Ile Cys Ala Gln Val Leu Pro Tyr Leu
      85              90              95
Ser Gln Glu His Gln Gln Gln Val Leu Gly Ala Ile Glu Arg Ala Lys
      100              105              110
Gln Val Thr Ala Pro Glu Leu Asn Ser Ile Ile Arg Gln Gln Leu Gln
      115              120              125
Ala His Gln Leu Ser Gln Leu Gln Ala Leu Ala Leu Pro Leu Thr Pro
      130              135              140
Leu Pro Val Gly Leu Gln Pro Pro Ser Leu Pro Ala Val Ser Ala Gly
      145              150              155              160
Thr Gly Leu Leu Ser Leu Ser Ala Leu Gly Ser Gln Ala His Leu Ser
      165              170              175
Lys Glu Asp Lys Asn Gly His Asp Gly Asp Thr His Gln Glu Asp Asp
      180              185              190
Gly Glu Lys Ser Asp
      195

```

<210> 89

<211> 739

<212> PRT

<213> Homo sapiens

032796-132.ST25

<400> 89

Gly Asp Lys Glu Pro Thr Glu Thr Ile Gly Asp Leu Ser Ile Cys Leu
 1 5 10 15
 Asp Gly Leu Gln Leu Glu Ser Glu Val Val Thr Asn Gly Glu Thr Thr
 20 25 30
 Cys Ser Glu Ser Ala Ser Gln Asn Asp Asp Gly Ser Arg Ser Lys Asp
 35 40 45
 Glu Thr Arg Val Ser Thr Asn Gly Ser Asp Asp Pro Glu Asp Ala Gly
 50 55 60
 Ala Gly Glu Asn Arg Arg Val Ser Gly Asn Asn Ser Pro Ser Leu Ser
 65 70 75 80
 Asn Gly Gly Phe Lys Pro Ser Arg Pro Pro Arg Pro Ser Arg Pro Pro
 85 90 95
 Pro Pro Thr Pro Arg Arg Pro Ala Ser Val Asn Gly Ser Pro Ser Ala
 100 105 110
 Thr Ser Glu Ser Asp Gly Ser Ser Thr Gly Ser Leu Pro Pro Thr Asn
 115 120 125
 Thr Asn Thr Asn Thr Ser Glu Gly Ala Thr Ser Gly Leu Ile Ile Pro
 130 135 140
 Leu Thr Ile Ser Gly Gly Ser Gly Pro Arg Pro Leu Asn Pro Val Thr
 145 150 155 160
 Gln Ala Pro Leu Pro Pro Gly Trp Glu Gln Arg Val Asp Gln His Gly
 165 170 175
 Arg Val Tyr Tyr Val Asp His Val Glu Lys Arg Thr Thr Trp Asp Arg
 180 185 190
 Pro Glu Pro Leu Pro Pro Gly Trp Glu Arg Arg Val Asp Asn Met Gly
 195 200 205
 Arg Ile Tyr Tyr Val Asp His Phe Thr Arg Thr Thr Thr Trp Gln Arg
 210 215 220
 Pro Thr Leu Glu Ser Val Arg Asn Tyr Glu Gln Trp Gln Leu Gln Arg
 225 230 235 240
 Ser Gln Leu Gln Gly Ala Met Gln Gln Phe Asn Gln Arg Phe Ile Tyr
 245 250 255
 Gly Asn Gln Asp Leu Phe Ala Thr Ser Gln Ser Lys Glu Phe Asp Pro
 260 265 270
 Leu Gly Pro Leu Pro Pro Gly Trp Glu Lys Arg Thr Asp Ser Asn Gly
 275 280 285
 Arg Val Tyr Phe Val Asn His Asn Thr Arg Ile Thr Gln Trp Glu Asp
 290 295 300
 Pro Arg Ser Gln Gly Gln Leu Asn Glu Lys Pro Leu Pro Glu Gly Trp
 305 310 315 320
 Glu Met Arg Phe Thr Val Asp Gly Ile Pro Tyr Phe Val Asp His Asn
 325 330 335
 Arg Arg Thr Thr Thr Tyr Ile Asp Pro Arg Thr Gly Lys Ser Ala Leu
 340 345 350
 Asp Asn Gly Pro Gln Ile Ala Tyr Val Arg Asp Phe Lys Ala Lys Val
 355 360 365
 Gln Tyr Phe Arg Phe Trp Cys Gln Gln Leu Ala Met Pro Gln His Ile
 370 375 380
 Lys Ile Thr Val Thr Arg Lys Thr Leu Phe Glu Asp Ser Phe Gln Gln
 385 390 395 400
 Ile Met Ser Phe Ser Pro Gln Asp Leu Arg Arg Arg Leu Trp Val Ile
 405 410 415
 Phe Pro Gly Glu Gly Leu Asp Tyr Gly Gly Val Ala Arg Glu Trp
 420 425 430
 Phe Phe Leu Leu Ser His Glu Val Leu Asn Pro Met Tyr Cys Leu Phe

032796-132.ST25

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      435      440      445
Glu Tyr Ala Gly Lys Asp Asn Tyr Cys Leu Gln Ile Asn Pro Ala Ser
  450      455      460
Tyr Ile Asn Pro Asp His Leu Lys Tyr Phe Arg Phe Ile Gly Arg Phe
  465      470      475      480
Ile Ala Met Ala Leu Phe His Gly Lys Phe Ile Asp Thr Gly Phe Ser
      485      490      495
Leu Pro Phe Tyr Lys Arg Ile Leu Asn Lys Pro Val Gly Leu Lys Asp
      500      505      510
Leu Glu Ser Ile Asp Pro Glu Phe Tyr Asn Ser Leu Ile Trp Val Lys
      515      520      525
Glu Asn Asn Ile Glu Glu Cys Asp Leu Glu Met Tyr Phe Ser Val Asp
  530      535      540
Lys Glu Ile Leu Gly Glu Ile Lys Ser His Asp Leu Lys Pro Asn Gly
  545      550      555      560
Gly Asn Ile Leu Val Thr Glu Glu Asn Lys Glu Glu Tyr Ile Arg Met
      565      570      575
Val Ala Glu Trp Arg Leu Ser Arg Gly Val Glu Glu Gln Thr Gln Ala
      580      585      590
Phe Phe Glu Gly Phe Asn Glu Ile Leu Pro Gln Gln Tyr Leu Gln Tyr
  595      600      605
Phe Asp Ala Lys Glu Leu Glu Val Leu Leu Cys Gly Met Gln Glu Ile
  610      615      620
Asp Leu Asn Asp Trp Gln Arg His Ala Ile Tyr Arg His Tyr Ala Arg
  625      630      635      640
Thr Ser Lys Gln Ile Met Trp Phe Trp Gln Phe Val Lys Glu Ile Asp
      645      650      655
Asn Glu Lys Arg Met Arg Leu Leu Gln Phe Val Thr Gly Thr Cys Arg
      660      665      670
Leu Pro Val Gly Gly Phe Ala Asp Leu Met Gly Ser Asn Gly Pro Gln
      675      680      685
Lys Phe Cys Ile Glu Lys Val Gly Lys Glu Asn Trp Leu Pro Arg Ser
  690      695      700
His Thr Cys Phe Asn Arg Leu Asp Leu Pro Pro Tyr Lys Ser Tyr Glu
  705      710      715      720
Gln Leu Lys Glu Lys Leu Leu Phe Ala Ile Glu Glu Thr Glu Gly Phe
      725      730      735
Gly Gln Glu

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<210> 90

<211> 431

<212> PRT

<213> Homo sapiens

<400> 90

```

Gly Pro Pro Pro Thr Arg Ala Leu Pro Leu Pro Gln Ser Leu Pro Pro
  1      5      10      15
Asp Phe Arg Leu Glu Pro Thr Ala Pro Ala Leu Ser Pro Arg Ser Ser
      20      25      30
Phe Ala Ser Ser Ser Ala Ser Asp Ala Ser Lys Pro Ser Ser Pro Arg
      35      40      45
Gly Ser Leu Leu Leu Asp Gly Ala Gly Ala Gly Gly Ala Gly Gly Ser
  50      55      60
Arg Pro Cys Ser Asn Arg Thr Ser Gly Ile Ser Met Gly Tyr Asp Gln
  65      70      75      80
Arg His Gly Ser Pro Leu Pro Ala Gly Pro Cys Leu Phe Gly Pro Pro

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032796-132.ST25

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      85              90              95
Leu Ala Gly Ala Pro Ala Gly Tyr Ser Pro Gly Gly Val Pro Ser Ala
      100              105              110
Tyr Pro Glu Leu His Ala Ala Leu Asp Arg Leu Tyr Ala Gln Arg Pro
      115              120              125
Ala Gly Phe Gly Cys Gln Glu Ser Arg His Ser Tyr Pro Pro Ala Leu
      130              135              140
Gly Ser Pro Gly Ala Leu Ala Gly Ala Arg Val Gly Ala Ala Gly Pro
      145              150              155              160
Leu Glu Arg Arg Gly Ala Gln Pro Gly Arg His Ser Val Thr Gly Tyr
      165              170              175
Gly Asp Cys Ala Val Gly Ala Arg Tyr Gln Asp Glu Leu Thr Ala Leu
      180              185              190
Leu Arg Leu Thr Val Gly Thr Gly Gly Arg Glu Ala Gly Ala Arg Gly
      195              200              205
Glu Pro Ser Gly Ile Glu Pro Ser Gly Leu Glu Glu Pro Pro Gly Pro
      210              215              220
Phe Val Pro Glu Ala Ala Arg Ala Arg Met Arg Glu Pro Glu Ala Arg
      225              230              235              240
Glu Asp Tyr Phe Gly Thr Cys Ile Lys Cys Asn Lys Gly Ile Tyr Gly
      245              250              255
Gln Ser Asn Ala Cys Gln Ala Leu Asp Ser Leu Tyr His Thr Gln Cys
      260              265              270
Phe Val Cys Cys Ser Cys Gly Arg Thr Leu Arg Cys Lys Ala Phe Tyr
      275              280              285
Ser Val Asn Gly Ser Val Tyr Cys Glu Glu Asp Tyr Leu Phe Ser Gly
      290              295              300
Phe Gln Glu Ala Ala Glu Lys Cys Cys Val Cys Gly His Leu Ile Leu
      305              310              315              320
Glu Lys Ile Leu Gln Ala Met Gly Lys Ser Tyr His Pro Gly Cys Phe
      325              330              335
Arg Cys Ile Val Cys Asn Lys Cys Leu Asp Gly Ile Pro Phe Thr Val
      340              345              350
Asp Phe Ser Asn Gln Val Tyr Cys Val Thr Asp Tyr His Lys Asn Tyr
      355              360              365
Ala Pro Lys Cys Ala Ala Cys Gly Gln Pro Ile Leu Pro Ser Glu Gly
      370              375              380
Cys Glu Asp Ile Val Arg Val Ile Ser Met Asp Arg Asp Tyr His Phe
      385              390              395              400
Glu Cys Tyr His Cys Glu Asp Cys Arg Met Gln Leu Ser Asp Glu Glu
      405              410              415
Gly Cys Cys Cys Phe Pro Leu Asp Gly His Leu Leu Cys His Gly
      420              425              430

```

<210> 91

<211> 900

<212> PRT

<213> Homo sapiens

<400> 91

```

Gly Pro Gly Ser Arg His His Arg Ala Arg Asp Arg Leu Ile His Phe
 1              5              10              15
Gly Ala Val Ser Thr Asp Val Leu Gly Cys Ser Ala His Cys Ser Leu
      20              25              30
Thr Gln Ser Pro Lys Met Asn Ile Gln Glu Gln Gly Phe Pro Leu Asp
      35              40              45
Leu Gly Ala Ser Phe Thr Glu Asp Ala Pro Arg Pro Pro Val Pro Gly

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032796-132.ST25

50		55		60
Glu Glu Gly Glu Leu Val Ser Thr Asp Pro Arg Pro Ala Ser Tyr Ser				
65		70		75
Phe Cys Ser Gly Lys Gly Val Gly Ile Lys Gly Glu Thr Ser Thr Ala				
	85		90	
Thr Pro Arg Arg Ser Asp Leu Asp Leu Gly Tyr Glu Pro Glu Gly Ser				
	100		105	
Ala Ser Pro Thr Pro Pro Tyr Leu Lys Trp Ala Glu Ser Leu His Ser				
	115		120	
Leu Leu Asp Asp Gln Asp Gly Ile Ser Leu Phe Arg Thr Phe Leu Lys				
	130		135	
Gln Glu Gly Cys Ala Asp Leu Leu Asp Phe Trp Phe Ala Cys Thr Gly				
145		150		155
Phe Arg Lys Leu Glu Pro Cys Asp Ser Asn Glu Glu Lys Arg Leu Lys				
	165		170	
Leu Ala Arg Ala Ile Tyr Arg Lys Tyr Ile Leu Asp Asn Asn Gly Ile				
	180		185	
Val Ser Arg Gln Thr Lys Pro Ala Thr Lys Ser Phe Ile Lys Gly Cys				
	195		200	
Ile Met Lys Gln Leu Ile Asp Pro Ala Met Phe Asp Gln Ala Gln Thr				
	210		215	
Glu Ile Gln Ala Thr Met Glu Glu Asn Thr Tyr Pro Ser Phe Leu Lys				
225		230		235
Ser Asp Ile Tyr Leu Glu Tyr Thr Arg Thr Gly Ser Glu Ser Pro Lys				
	245		250	
Val Cys Ser Asp Gln Ser Ser Gly Ser Gly Thr Gly Lys Gly Ile Ser				
	260		265	
Gly Tyr Leu Pro Thr Leu Asn Glu Asp Glu Glu Trp Lys Cys Asp Gln				
	275		280	
Asp Met Asp Glu Asp Asp Gly Arg Asp Ala Ala Pro Pro Gly Arg Leu				
	290		295	
Pro Gln Lys Leu Leu Leu Glu Thr Ala Ala Pro Arg Val Ser Ser Ser				
305		310		315
Arg Arg Tyr Ser Glu Gly Arg Glu Phe Arg Tyr Gly Ser Trp Arg Glu				
	325		330	
Pro Val Asn Pro Tyr Tyr Val Asn Ala Gly Tyr Ala Leu Ala Pro Ala				
	340		345	
Thr Ser Ala Asn Asp Ser Glu Gln Ser Leu Ser Ser Asp Ala Asp				
	355		360	
Thr Leu Ser Leu Thr Asp Ser Ser Val Asp Gly Ile Pro Pro Tyr Arg				
	370		375	
Ile Arg Lys Gln His Arg Arg Glu Met Gln Glu Ser Ala Gln Val Asn				
385		390		395
Gly Arg Val Pro Leu Pro His Ile Pro Arg Thr Tyr Arg Val Pro Lys				
	405		410	
Glu Val Arg Val Glu Pro Gln Lys Phe Ala Glu Glu Leu Ile His Arg				
	420		425	
Leu Glu Ala Val Gln Arg Thr Arg Glu Ala Glu Glu Lys Leu Glu Glu				
	435		440	
Arg Leu Lys Arg Val Arg Met Glu Glu Glu Gly Glu Asp Gly Asp Pro				
	450		455	
Ser Ser Gly Pro Pro Gly Pro Cys His Lys Leu Pro Pro Ala Pro Ala				
465		470		475
Trp His His Phe Pro Pro Arg Leu Cys Trp Thr Trp Ala Cys Ala Gly				
	485		490	
Leu Arg Asp Ala His Glu Glu Asn Pro Glu Ser Ile Leu Asp Glu His				
	500		505	
			510	

032796-132.ST25

Val Gln Arg Val Leu Arg Thr Thr Gly Arg Gln Ser Pro Gly Pro Gly
 515 520 525
 His Arg Ser Pro Asp Ser Gly His Val Ala Lys Met Pro Val Ala Leu
 530 535 540
 Gly Gly Ala Ala Ser Gly His Gly Lys His Val Pro Lys Ser Gly Ala
 545 550 555 560
 Lys Leu Asp Ala Ala Gly Leu His His His Arg His Val His His His
 565 570 575
 Val His His Ser Thr Ala Arg Pro Lys Glu Gln Val Glu Ala Glu Ala
 580 585 590
 Thr Arg Arg Ala Gln Ser Ser Phe Ala Trp Gly Leu Glu Pro His Ser
 595 600 605
 His Gly Ala Arg Ser Arg Gly Tyr Ser Glu Ser Val Gly Ala Ala Pro
 610 615 620
 Asn Ala Ser Asp Gly Leu Ala His Ser Gly Lys Val Gly Val Ala Cys
 625 630 635 640
 Lys Arg Asn Ala Lys Lys Ala Glu Ser Gly Lys Ser Ala Ser Thr Glu
 645 650 655
 Val Pro Gly Ala Ser Glu Asp Ala Glu Lys Asn Gln Lys Ile Met Gln
 660 665 670
 Trp Ile Ile Glu Gly Glu Lys Glu Ile Ser Arg His Arg Arg Thr Gly
 675 680 685
 His Gly Ser Ser Gly Thr Arg Lys Pro Gln Pro His Glu Asn Ser Arg
 690 695 700
 Pro Leu Ser Leu Glu His Pro Trp Ala Gly Pro Gln Leu Arg Thr Ser
 705 710 715 720
 Val Gln Pro Ser His Leu Phe Ile Gln Asp Pro Thr Met Pro Pro His
 725 730 735
 Pro Ala Pro Asn Pro Leu Thr Gln Leu Glu Glu Ala Arg Arg Arg Leu
 740 745 750
 Glu Glu Glu Glu Lys Arg Ala Ser Arg Ala Pro Ser Lys Gln Arg Tyr
 755 760 765
 Val Gln Glu Val Met Arg Arg Gly Arg Ala Cys Val Arg Pro Ala Cys
 770 775 780
 Ala Pro Val Leu His Val Val Pro Ala Val Ser Asp Met Glu Leu Ser
 785 790 795 800
 Glu Thr Glu Thr Arg Ser Gln Arg Lys Val Gly Gly Gly Ser Ala Gln
 805 810 815
 Pro Cys Asp Ser Ile Val Val Ala Tyr Tyr Phe Cys Gly Glu Pro Ile
 820 825 830
 Pro Tyr Arg Thr Leu Val Arg Gly Arg Ala Val Thr Leu Gly Gln Phe
 835 840 845
 Lys Glu Leu Leu Thr Lys Lys Gly Ser Tyr Arg Tyr Tyr Phe Lys Lys
 850 855 860
 Val Ser Asp Glu Phe Asp Cys Gly Val Val Phe Glu Glu Val Arg Glu
 865 870 875 880
 Asp Glu Ala Val Leu Pro Val Phe Glu Glu Lys Ile Ile Gly Lys Val
 885 890 895
 Glu Lys Val Asp
 900

<210> 92

<211> 591

<212> PRT

<213> Homo sapiens

<400> 92

032796-132.ST25

Met Val Pro Val Ala Val Thr Ala Ala Val Ala Pro Val Leu Ser Ile
 1 5 10 15
 Asn Ser Asp Phe Ser Asp Leu Arg Glu Ile Lys Lys Gln Leu Leu Leu
 20 25 30
 Ile Ala Gly Leu Thr Arg Glu Arg Gly Leu Leu His Ser Ser Lys Trp
 35 40 45
 Ser Ala Glu Leu Ala Phe Ser Leu Pro Ala Leu Pro Leu Ala Glu Leu
 50 55 60
 Gln Pro Pro Pro Pro Ile Thr Glu Glu Asp Ala Gln Asp Met Asp Ala
 65 70 75 80
 Tyr Thr Leu Ala Lys Ala Tyr Phe Asp Val Lys Glu Tyr Asp Arg Ala
 85 90 95
 Ala His Phe Leu His Gly Cys Asn Ser Lys Lys Ala Tyr Phe Leu Tyr
 100 105 110
 Met Tyr Ser Arg Tyr Leu Ser Gly Glu Lys Lys Lys Asp Asp Glu Thr
 115 120 125
 Val Asp Ser Leu Gly Pro Leu Glu Lys Gly Gln Val Lys Asn Glu Ala
 130 135 140
 Leu Arg Glu Leu Arg Val Glu Leu Ser Lys Lys His Gln Ala Arg Glu
 145 150 155 160
 Leu Asp Gly Phe Gly Leu Tyr Leu Tyr Gly Val Val Leu Arg Lys Leu
 165 170 175
 Asp Leu Val Lys Glu Ala Ile Asp Val Phe Val Glu Ala Thr His Val
 180 185 190
 Leu Pro Leu His Trp Gly Ala Trp Leu Glu Leu Cys Asn Leu Ile Thr
 195 200 205
 Asp Lys Glu Met Leu Lys Phe Leu Ser Leu Pro Asp Thr Trp Met Lys
 210 215 220
 Glu Phe Phe Leu Ala His Ile Tyr Thr Glu Leu Gln Leu Ile Glu Glu
 225 230 235 240
 Ala Leu Gln Lys Tyr Gln Asn Leu Ile Asp Val Gly Phe Ser Lys Ser
 245 250 255
 Ser Tyr Ile Val Ser Gln Ile Ala Val Ala Tyr His Asn Ile Arg Asp
 260 265 270
 Ile Asp Lys Ala Leu Ser Ile Phe Asn Glu Leu Arg Lys Gln Asp Pro
 275 280 285
 Tyr Arg Ile Glu Asn Met Asp Thr Phe Ser Asn Leu Leu Tyr Val Arg
 290 295 300
 Ser Met Lys Ser Glu Leu Ser Tyr Leu Ala His Asn Leu Cys Glu Ile
 305 310 315 320
 Asp Lys Tyr Arg Val Glu Thr Cys Cys Val Ile Gly Asn Tyr Tyr Ser
 325 330 335
 Leu Arg Ser Gln His Glu Lys Ala Ala Leu Tyr Phe Gln Arg Ala Leu
 340 345 350
 Lys Leu Asn Pro Arg Tyr Leu Gly Ala Trp Thr Leu Met Gly His Glu
 355 360 365
 Tyr Met Glu Met Lys Asn Thr Ser Ala Ala Ile Gln Ala Tyr Arg His
 370 375 380
 Ala Ile Glu Val Asn Lys Arg Asp Tyr Arg Ala Trp Tyr Gly Leu Gly
 385 390 395 400
 Gln Thr Tyr Glu Ile Leu Lys Met Pro Phe Tyr Cys Leu Tyr Tyr Tyr
 405 410 415
 Arg Arg Ala His Gln Leu Arg Pro Asn Asp Ser Arg Met Leu Val Ala
 420 425 430
 Leu Gly Glu Cys Tyr Glu Lys Leu Asn Gln Leu Val Glu Ala Lys Lys
 435 440 445
 Cys Tyr Trp Arg Ala Tyr Ala Val Gly Asp Val Glu Lys Met Ala Leu

032796-132.ST25

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      450              455              460
Val Lys Leu Ala Lys Leu His Glu Gln Leu Thr Glu Ser Glu Gln Ala
465              470              475              480
Ala Gln Cys Tyr Ile Lys Tyr Ile Gln Asp Ile Tyr Ser Cys Gly Glu
      485              490              495
Ile Val Glu His Leu Glu Glu Ser Thr Ala Phe Arg Tyr Leu Ala Gln
      500              505              510
Tyr Tyr Phe Lys Cys Lys Leu Trp Asp Glu Ala Ser Thr Cys Ala Gln
      515              520              525
Lys Cys Cys Ala Phe Asn Asp Thr Arg Glu Glu Gly Lys Ala Leu Leu
      530              535              540
Arg Gln Ile Leu Gln Leu Arg Asn Gln Gly Glu Thr Pro Thr Thr Glu
      545              550              555              560
Val Pro Ala Pro Phe Leu Pro Ala Ser Leu Ser Ala Asn Asn Thr
      565              570              575
Pro Thr Arg Arg Val Ser Pro Leu Asn Leu Ser Ser Val Thr Pro
      580              585              590

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<210> 93
 <211> 914
 <212> PRT
 <213> Homo sapiens

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<400> 93
Val Tyr Gln Val Leu Leu Val Gly Ser Thr Leu Leu Lys Glu Val Pro
  1              5              10              15
Ser Gly Leu Gln Leu Glu Gln Leu Pro Ser Gln Ser Leu Leu Thr His
      20              25              30
Ile Pro Thr Ala Gly Leu Pro Thr Ser Leu Gly Gly Gly Leu Pro Tyr
      35              40              45
Cys His Gln Ala Trp Leu Asp Phe Arg Arg Arg Leu Glu Ala Leu Leu
      50              55              60
Gln Asn Cys Gln Ala Ala Cys Ala Leu Leu Gln Gly Ala Ile Glu Ser
      65              70              75              80
Val Lys Ala Val Pro Gln Pro Met Glu Pro Gly Glu Val Gly Gln Leu
      85              90              95
Leu Gln Gln Thr Glu Val Leu Met Gln Gln Val Leu Asp Ser Pro Trp
      100             105             110
Leu Ala Trp Leu Gln Cys Gln Gly Gly Arg Glu Leu Thr Trp Leu Lys
      115             120             125
Gln Glu Val Pro Glu Val Thr Leu Ser Pro Asp Tyr Arg Thr Ala Met
      130             135             140
Asp Lys Ala Asp Glu Leu Tyr Asp Arg Val Asp Gly Leu Leu His Gln
      145             150             155             160
Leu Thr Leu Gln Ser Asn Gln Arg Ile Gln Ala Leu Glu Leu Val Gln
      165             170             175
Thr Leu Glu Ala Arg Glu Ser Gly Leu His Gln Ile Glu Val Trp Leu
      180             185             190
Gln Gln Val Gly Trp Pro Ala Leu Glu Glu Ala Gly Glu Pro Ser Leu
      195             200             205
Asp Met Leu Leu Gln Ala Gln Gly Ser Phe Gln Glu Leu Tyr Gln Val
      210             215             220
Ala Gln Glu Gln Val Arg Gln Gly Glu Lys Phe Leu Gln Pro Leu Thr
      225             230             235             240
Gly Trp Glu Ala Ala Glu Leu Asp Pro Pro Gly Ala Arg Phe Leu Ala
      245             250             255
Leu Arg Ala Gln Leu Thr Glu Phe Ser Arg Ala Leu Ala Gln Arg Cys

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032796-132.ST25

260										265					270					
Gln	Arg	Leu	Ala	Asp	Ala	Glu	Arg	Leu	Phe	Gln	Leu	Phe	Arg	Glu	Ala					
275										280					285					
Leu	Thr	Trp	Ala	Glu	Glu	Gly	Gln	Arg	Val	Leu	Ala	Glu	Leu	Glu	Gln					
290										295					300					
Glu	Arg	Pro	Gly	Val	Val	Leu	Gln	Gln	Leu	Gln	Leu	His	Trp	Thr	Arg					
305										310					315					
His	Pro	Asp	Leu	Pro	Pro	Ala	His	Phe	Arg	Lys	Met	Trp	Ala	Leu	Ala					
325										330					335					
Thr	Gly	Leu	Gly	Ser	Glu	Ala	Ile	Arg	Gln	Glu	Cys	Arg	Trp	Ala	Trp					
340										345					350					
Ala	Arg	Cys	Gln	Asp	Thr	Trp	Leu	Ala	Leu	Asp	Gln	Lys	Leu	Glu	Ala					
355										360					365					
Ser	Leu	Lys	Leu	Pro	Pro	Val	Gly	Ser	Thr	Ala	Ser	Leu	Cys	Val	Ser					
370										375					380					
Gln	Val	Pro	Ala	Ala	Pro	Ala	His	Pro	Pro	Leu	Arg	Lys	Ala	Tyr	Ser					
385										390					395					
Phe	Asp	Arg	Asn	Leu	Gly	Gln	Ser	Leu	Ser	Glu	Pro	Ala	Cys	His	Cys					
405										410					415					
His	His	Ala	Ala	Thr	Ile	Ala	Ala	Cys	Arg	Arg	Pro	Glu	Ala	Gly	Gly					
420										425					430					
Gly	Ala	Leu	Pro	Gln	Ala	Ser	Pro	Thr	Val	Pro	Pro	Pro	Gly	Ser	Ser					
435										440					445					
Asp	Pro	Arg	Ser	Leu	Asn	Arg	Leu	Gln	Leu	Val	Leu	Ala	Glu	Met	Val					
450										455					460					
Ala	Thr	Glu	Arg	Glu	Tyr	Val	Arg	Ala	Leu	Glu	Tyr	Thr	Met	Glu	Asn					
465										470					475					
Tyr	Phe	Pro	Glu	Leu	Asp	Arg	Pro	Asp	Val	Pro	Gln	Gly	Leu	Arg	Gly					
485										490					495					
Gln	Arg	Ala	His	Leu	Phe	Gly	Asn	Leu	Glu	Lys	Leu	Arg	Asp	Phe	His					
500										505					510					
Cys	His	Phe	Phe	Leu	Arg	Glu	Leu	Glu	Ala	Cys	Thr	Arg	His	Pro	Pro					
515										520					525					
Arg	Val	Ala	Tyr	Ala	Phe	Leu	Arg	His	Arg	Val	Gln	Phe	Gly	Met	Tyr					
530										535					540					
Ala	Leu	Tyr	Ser	Lys	Asn	Lys	Pro	Arg	Ser	Asp	Ala	Leu	Met	Ser	Ser					
545										550					555					
Tyr	Gly	His	Thr	Phe	Lys	Asp	Lys	Gln	Gln	Ala	Leu	Gly	Asp	His						
565										570					575					
Leu	Asp	Leu	Ala	Ser	Tyr	Leu	Leu	Lys	Pro	Ile	Gln	Arg	Met	Gly	Lys					
580										585					590					
Tyr	Ala	Leu	Leu	Leu	Gln	Glu	Leu	Ala	Arg	Ala	Cys	Gly	Gly	Pro	Thr					
595										600					605					
Gln	Glu	Leu	Ser	Ala	Leu	Arg	Glu	Ala	Gln	Ser	Leu	Val	His	Phe	Gln					
610										615					620					
Leu	Arg	His	Gly	Asn	Asp	Leu	Leu	Ala	Met	Asp	Ala	Ile	Gln	Gly	Cys					
625										630					635					
Asp	Val	Asn	Leu	Lys	Glu	Gln	Gly	Gln	Leu	Val	Arg	Gln	Asp	Glu	Phe					
645										650					655					
Val	Val	Arg	Thr	Gly	Arg	His	Lys	Ser	Val	Arg	Arg	Ile	Phe	Leu	Phe					
660										665					670					
Glu	Glu	Leu	Leu	Leu	Phe	Ser	Lys	Pro	Arg	His	Gly	Pro	Thr	Gly	Val					
675										680					685					
Asp	Thr	Phe	Ala	Tyr	Lys	Arg	Ser	Phe	Lys	Met	Ala	Asp	Leu	Gly	Leu					
690										695					700					
Thr	Glu	Cys	Cys	Gly	Asn	Ser	Asn	Leu	Arg	Phe	Glu	Ile	Trp	Phe	Arg					
705										710					715					
															720					

032796-132.ST25

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Arg Arg Lys Ala Arg Asp Thr Phe Val Leu Gln Ala Ser Ser Leu Ala
      725              730              735
Ile Lys Gln Ala Trp Thr Ala Asp Ile Ser His Leu Leu Trp Arg Gln
      740              745              750
Ala Val His Asn Lys Glu Val Arg Met Ala Glu Met Val Ser Met Gly
      755              760              765
Val Gly Asn Lys Ala Phe Arg Asp Ile Ala Pro Ser Glu Glu Ala Ile
      770              775              780
Asn Asp Arg Thr Val Asn Tyr Val Leu Lys Cys Arg Glu Val Arg Ser
      785              790              795              800
Arg Ala Ser Ile Ala Val Ala Pro Phe Asp His Asp Ser Leu Tyr Leu
      805              810              815
Gly Ala Ser Asn Ser Leu Pro Gly Asp Pro Ala Ser Cys Ser Val Leu
      820              825              830
Gly Ser Leu Asn Leu His Leu Tyr Arg Asp Pro Ala Leu Leu Gly Leu
      835              840              845
Arg Cys Pro Leu Tyr Pro Ser Phe Leu Glu Glu Ala Ala Leu Glu Ala
      850              855              860
Glu Ala Glu Leu Gly Gly Gln Pro Ser Leu Thr Ala Glu Asp Ser Glu
      865              870              875              880
Ile Ser Ser Gln Cys Pro Ser Ala Ser Gly Ser Ser Gly Ser Asp Ser
      885              890              895
Ser Cys Val Ser Gly Gln Ala Leu Gly Arg Gly Leu Glu Asp Leu Pro
      900              905              910
Cys Val

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<210> 94

<211> 277

<212> PRT

<213> Homo sapiens

<400> 94

```

Leu Asn Tyr Leu Leu Glu Ser Arg Leu Glu Ala Ala Ala His Cys Ala
  1              5              10              15
Leu Lys Gln Gly Ile Ala Thr Ala Ser Leu Leu Pro Ala Gln Leu Gln
  20              25              30
Pro Ala Val Leu Thr Val Val Thr Cys His Val Val Val Ser Val His
  35              40              45
Gly His His Thr Asp Gly Cys Leu Ala Ala Leu Cys Arg Glu Asp Arg
  50              55              60
Thr Gly Thr Gly Gly Ala Phe Trp Cys Lys Asn Arg Val Ile Val Ser
  65              70              75              80
His Ala Val Asp Val Val Leu His Val His Gly Glu Gly Asn Pro Val
  85              90              95
Gln Ala Leu Ile Ala His Gly Ala Pro Glu Ala Ala Trp Val Val Gly
  100             105             110
Leu Ala Gln Gly Leu Gln Asp His Phe His Asp Glu Met Ser Thr His
  115             120             125
Ala Ala Phe Val Gly Arg Leu Leu Glu Pro Gly Val Gln Glu Val Leu
  130             135             140
Leu Ala Val His Phe Leu Thr His Val Val Glu Arg Leu Pro Thr Glu
  145             150             155             160
Ser Ser Pro Thr Arg Val Ala Gly Glu Ala Val Ser Val Ile Lys Thr
  165             170             175
Pro His Cys Leu Ala Arg Leu Leu Gly Ser Val Asp Ala Lys Pro Thr
  180             185             190

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032796-132.ST25

Leu Asp Ala Asn Ala Glu Val Val Pro Arg Arg Ala Arg Leu Glu Arg
 195 200 205
 Pro Leu Gln Leu Pro Gly Glu Arg Leu Gln Pro Pro Leu Gly Arg Ala
 210 215 220
 Trp Ala Ala Leu Pro Ala Arg Gly Gln Arg Glu Cys Arg Gln Arg Glu
 225 230 235 240
 Gly Gly Arg Pro Arg Arg Leu Arg Gly Ala Ser Gly Arg Gly Ala Gly
 245 250 255
 Ala Gly Arg Glu Glu Val Ser Val Gly Phe Ser Ala Gln Trp Glu Phe
 260 265 270
 Gly Ser Gly Arg His
 275

<210> 95

<211> 1120

<212> PRT

<213> Homo sapiens

<400> 95

Met Trp Arg Val Lys Lys Leu Ser Leu Ser Leu Ser Pro Ser Pro Gln
 1 5 10 15
 Thr Gly Lys Pro Ser Met Arg Thr Pro Leu Arg Glu Leu Thr Leu Gln
 20 25 30
 Pro Gly Ala Leu Thr Thr Ser Gly Lys Arg Ser Pro Ala Cys Ser Ser
 35 40 45
 Leu Thr Pro Ser Leu Cys Lys Leu Gly Leu Gln Glu Gly Ser Asn Asn
 50 55 60
 Ser Ser Pro Val Asp Phe Val Asn Asn Lys Arg Thr Asp Leu Ser Ser
 65 70 75 80
 Glu His Phe Ser His Ser Ser Lys Trp Leu Glu Thr Cys Gln His Glu
 85 90 95
 Ser Asp Glu Gln Pro Leu Asp Pro Ile Pro Gln Ile Ser Ser Thr Pro
 100 105 110
 Lys Thr Ser Glu Glu Ala Val Asp Pro Leu Gly Asn Tyr Met Val Lys
 115 120 125
 Thr Ile Val Leu Val Pro Ser Pro Leu Gly Gln Gln Gln Asp Met Ile
 130 135 140
 Phe Glu Ala Arg Leu Asp Thr Met Ala Glu Thr Asn Ser Ile Ser Leu
 145 150 155 160
 Asn Gly Pro Leu Arg Thr Asp Asp Leu Val Arg Glu Glu Val Ala Pro
 165 170 175
 Cys Met Gly Asp Arg Phe Ser Glu Val Ala Ala Val Ser Glu Lys Pro
 180 185 190
 Ile Phe Gln Glu Ser Pro Ser His Leu Leu Glu Glu Ser Pro Pro Asn
 195 200 205
 Pro Cys Ser Glu Gln Leu His Cys Ser Lys Glu Ser Leu Ser Ser Arg
 210 215 220
 Thr Glu Ala Val Arg Glu Asp Leu Val Pro Ser Glu Ser Asn Ala Phe
 225 230 235 240
 Leu Pro Ser Ser Val Leu Trp Leu Ser Pro Ser Thr Ala Leu Ala Ala
 245 250 255
 Asp Phe Arg Val Asn His Val Asp Pro Glu Glu Glu Ile Val Glu His
 260 265 270
 Gly Ala Met Glu Glu Arg Glu Met Arg Phe Pro Thr His Pro Lys Glu
 275 280 285
 Ser Glu Thr Glu Asp Gln Ala Leu Val Ser Ser Val Glu Asp Ile Leu
 290 295 300

032796-132.ST25

Ser Thr Cys Leu Thr Pro Asn Leu Val Glu Met Glu Ser Gln Glu Ala
 305 310 315 320
 Pro Gly Pro Ala Val Glu Asp Val Gly Arg Ile Leu Gly Ser Asp Thr
 325 330 335
 Glu Ser Trp Met Ser Pro Leu Ala Trp Leu Glu Lys Gly Val Asn Thr
 340 345 350
 Ser Val Met Leu Glu Asn Leu Arg Gln Ser Leu Ser Leu Pro Ser Met
 355 360 365
 Leu Arg Asp Ala Ala Ile Gly Thr Thr Pro Phe Ser Thr Cys Ser Val
 370 375 380
 Gly Thr Trp Phe Thr Pro Ser Ala Pro Gln Glu Lys Ser Thr Asn Thr
 385 390 395 400
 Ser Gln Thr Gly Leu Val Gly Thr Lys His Ser Thr Ser Glu Thr Glu
 405 410 415
 Gln Leu Leu Cys Gly Arg Pro Pro Asp Leu Thr Ala Leu Ser Arg His
 420 425 430
 Asp Leu Glu Asp Asn Leu Leu Ser Ser Leu Val Ile Val Glu Phe Leu
 435 440 445
 Ser Arg Gln Leu Arg Asp Trp Lys Ser Gln Leu Ala Val Pro His Pro
 450 455 460
 Glu Thr Gln Asp Ser Ser Thr Gln Thr Asp Thr Ser His Ser Gly Ile
 465 470 475 480
 Thr Asn Lys Leu Gln His Leu Lys Glu Ser His Glu Met Gly Gln Ala
 485 490 495
 Leu Gln Gln Ala Arg Asn Val Met Gln Ser Trp Val Leu Ile Ser Lys
 500 505 510
 Glu Leu Ile Ser Leu Leu His Leu Ser Leu Leu His Leu Glu Glu Asp
 515 520 525
 Lys Thr Thr Val Asn Gln Glu Ser Arg Arg Ala Glu Thr Leu Val Cys
 530 535 540
 Cys Cys Phe Asp Leu Leu Lys Lys Leu Arg Ala Lys Leu Gln Ser Leu
 545 550 555 560
 Lys Ala Glu Arg Glu Glu Ala Arg His Arg Glu Glu Met Ala Leu Arg
 565 570 575
 Gly Lys Asp Ala Ala Glu Ile Val Leu Glu Ala Phe Cys Ala His Ala
 580 585 590
 Ser Gln Arg Ile Ser Gln Leu Glu Gln Asp Leu Ala Ser Met Arg Glu
 595 600 605
 Phe Arg Gly Leu Leu Lys Asp Ala Gln Thr Gln Leu Val Gly Leu His
 610 615 620
 Ala Lys Gln Glu Glu Leu Val Gln Gln Thr Val Ser Leu Thr Ser Thr
 625 630 635 640
 Leu Gln Gln Asp Trp Arg Ser Met Gln Leu Asp Tyr Thr Thr Trp Thr
 645 650 655
 Ala Leu Leu Ser Arg Ser Arg Gln Leu Thr Glu Lys Leu Thr Val Lys
 660 665 670
 Ser Gln Gln Ala Leu Gln Glu Arg Asp Val Ala Ile Glu Glu Lys Gln
 675 680 685
 Glu Val Ser Arg Val Leu Glu Gln Val Ser Ala Gln Leu Glu Glu Cys
 690 695 700
 Lys Gly Gln Thr Glu Gln Leu Glu Leu Glu Asn Ile Arg Leu Ala Thr
 705 710 715 720
 Asp Leu Arg Ala Gln Leu Gln Ile Leu Ala Asn Met Asp Ser Gln Leu
 725 730 735
 Lys Glu Leu Gln Ser Gln His Thr His Cys Ala Gln Asp Leu Ala Met
 740 745 750
 Lys Asp Glu Leu Leu Cys Gln Leu Thr Gln Ser Asn Glu Glu Gln Ala

032796-132.ST25

```

      755              760              765
Ala Gln Cys Val Lys Glu Glu Met Ala Leu Lys His Met Gln Ala Glu
770              775              780
Leu Gln Gln Gln Gln Ala Val Leu Ala Lys Glu Val Arg Asp Leu Lys
785              790              795              800
Glu Thr Leu Glu Phe Ala Asp Gln Glu Asn Gln Val Ala His Leu Glu
805              810              815
Leu Gly Gln Val Glu Cys Gln Leu Lys Thr Thr Leu Glu Val Leu Arg
820              825              830
Glu Arg Ser Leu Gln Cys Glu Asn Leu Lys Asp Thr Val Glu Asn Leu
835              840              845
Thr Ala Lys Leu Ala Ser Thr Ile Ala Asp Asn Gln Glu Gln Asp Leu
850              855              860
Glu Lys Thr Arg Gln Tyr Ser Gln Lys Leu Gly Leu Leu Thr Glu Gln
865              870              875              880
Leu Gln Ser Leu Thr Leu Phe Leu Gln Thr Lys Leu Lys Glu Lys Thr
885              890              895
Glu Gln Glu Thr Leu Leu Leu Ser Thr Ala Cys Pro Pro Thr Gln Glu
900              905              910
His Pro Leu Pro Asn Asp Arg Thr Phe Leu Gly Ser Ile Leu Thr Ala
915              920              925
Val Ala Asp Glu Glu Pro Glu Ser Thr Pro Val Pro Leu Leu Gly Ser
930              935              940
Asp Lys Ser Ala Phe Thr Arg Val Ala Ser Met Val Ser Leu Gln Pro
945              950              955              960
Ala Glu Thr Pro Gly Met Glu Glu Ser Leu Ala Glu Met Ser Ile Met
965              970              975
Thr Thr Glu Leu Gln Ser Leu Cys Ser Leu Leu Gln Glu Ser Lys Glu
980              985              990
Glu Ala Ile Arg Thr Leu Gln Arg Lys Ile Cys Glu Leu Gln Ala Arg
995              1000              1005
Leu Gln Ala Gln Glu Glu Gln His Gln Glu Val Gln Lys Ala Lys Glu
1010              1015              1020
Ala Asp Ile Glu Lys Leu Asn Gln Ala Leu Cys Leu Arg Tyr Lys Asn
1025              1030              1035              1040
Glu Lys Glu Leu Gln Glu Val Ile Gln Gln Asn Glu Lys Ile Leu Glu
1045              1050              1055
Gln Ile Asp Lys Ser Gly Glu Leu Ile Ser Leu Arg Glu Glu Val Thr
1060              1065              1070
His Leu Thr Arg Ser Leu Arg Arg Ala Glu Thr Glu Thr Lys Val Leu
1075              1080              1085
Gln Glu Ala Trp Gln Ala Ser Trp Thr Pro Thr Ala Ser Leu Trp Pro
1090              1095              1100
Pro Ile Gly Ser Arg Arg Lys Cys Gly Ser Leu Arg Arg Trp Thr Asn
1105              1110              1115              1120

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<210> 96

<211> 540

<212> PRT

<213> Homo sapiens

<400> 96

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Met Gly Thr Thr Ala Arg Ala Ala Leu Val Leu Thr Tyr Leu Ala Val
1      5      10      15
Ala Ser Ala Ala Ser Glu Gly Gly Phe Thr Ala Thr Gly Gln Arg Gln
20     25     30
Leu Arg Pro Glu His Phe Gln Glu Val Gly Tyr Ala Ala Pro Pro Ser

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032796-132.ST25

35	40	45
Pro Pro Leu Ser Arg Ser Leu Pro Met Asp His Pro Asp Ser Ser Gln		
50	55	60
His Gly Pro Pro Phe Glu Gly Gln Ser Gln Val Gln Pro Pro Pro Ser		
65	70	75
Gln Glu Ala Thr Pro Leu Gln Gln Glu Lys Leu Leu Pro Ala Gln Leu		
85	90	95
Pro Ala Glu Lys Glu Val Gly Pro Pro Leu Pro Gln Glu Ala Val Pro		
100	105	110
Leu Gln Lys Glu Leu Pro Ser Leu Gln His Pro Asn Glu Gln Lys Glu		
115	120	125
Gly Thr Pro Ala Pro Phe Gly Asp Gln Ser His Pro Glu Pro Glu Ser		
130	135	140
Trp Asn Ala Ala Gln His Cys Gln Gln Asp Arg Ser Gln Gly Gly Trp		
145	150	155
Gly His Arg Leu Asp Gly Phe Pro Pro Gly Arg Pro Ser Pro Asp Asn		
165	170	175
Leu Asn Gln Ile Cys Leu Pro Asn Arg Gln His Val Val Tyr Gly Pro		
180	185	190
Trp Asn Leu Pro Gln Ser Ser Tyr Ser His Leu Thr Arg Gln Gly Glu		
195	200	205
Thr Leu Asn Phe Leu Glu Ile Gly Tyr Ser Arg Cys Cys His Cys Arg		
210	215	220
Ser His Thr Asn Arg Leu Glu Cys Ala Lys Leu Val Trp Glu Glu Ala		
225	230	235
Met Ser Arg Phe Cys Glu Ala Glu Phe Ser Val Lys Thr Arg Pro His		
245	250	255
Trp Cys Cys Thr Arg Gln Gly Glu Ala Arg Phe Ser Cys Phe Gln Glu		
260	265	270
Glu Ala Pro Gln Pro His Tyr Gln Leu Arg Ala Cys Pro Ser His Gln		
275	280	285
Pro Asp Ile Ser Ser Gly Leu Glu Leu Pro Phe Pro Pro Gly Val Pro		
290	295	300
Thr Leu Asp Asn Ile Lys Asn Ile Cys His Leu Arg Arg Phe Arg Ser		
305	310	315
Val Pro Arg Asn Leu Pro Ala Thr Asp Pro Leu Gln Arg Glu Leu Leu		
325	330	335
Ala Leu Ile Gln Leu Glu Arg Glu Phe Gln Arg Cys Cys Arg Gln Gly		
340	345	350
Asn Asn His Thr Cys Thr Trp Lys Ala Trp Glu Asp Thr Leu Asp Lys		
355	360	365
Tyr Cys Asp Arg Glu Tyr Ala Val Lys Thr His His His Leu Cys Cys		
370	375	380
Arg His Pro Pro Ser Pro Thr Arg Asp Glu Cys Phe Ala Arg Arg Ala		
385	390	395
Pro Tyr Pro Asn Tyr Asp Arg Asp Ile Leu Thr Ile Asp Ile Ser Arg		
405	410	415
Val Thr Pro Asn Leu Met Gly His Leu Cys Gly Asn Gln Arg Val Leu		
420	425	430
Thr Lys His Lys His Ile Pro Gly Leu Ile His Asn Met Thr Ala Arg		
435	440	445
Cys Cys Asp Leu Pro Phe Pro Glu Gln Ala Cys Cys Ala Glu Glu Glu		
450	455	460
Lys Leu Thr Phe Ile Asn Asp Leu Cys Gly Pro Arg Arg Asn Ile Trp		
465	470	475
Arg Asp Pro Ala Leu Cys Cys Tyr Leu Ser Pro Gly Asp Glu Gln Val		
485	490	495

032796-132.ST25

Asn Cys Phe Asn Ile Asn Tyr Leu Arg Asn Val Ala Leu Val Ser Gly
 500 505 510
 Asp Thr Glu Asn Ala Lys Gly Gln Gly Glu Gln Gly Ser Thr Gly Gly
 515 520 525
 Thr Asn Ile Ser Ser Thr Ser Glu Pro Lys Glu Glu
 530 535 540

<210> 97
 <211> 462
 <212> PRT
 <213> Homo sapiens

<400> 97
 Met Gly Lys Glu Lys Thr His Ile Asn Ile Val Val Ile Gly His Val
 1 5 10 15
 Asp Ser Gly Lys Ser Thr Thr Thr Gly His Leu Ile Tyr Lys Cys Gly
 20 25 30
 Gly Ile Asp Lys Arg Thr Ile Glu Lys Phe Glu Lys Glu Ala Ala Glu
 35 40 45
 Met Gly Lys Gly Ser Phe Lys Tyr Ala Trp Val Leu Asp Lys Leu Lys
 50 55 60
 Ala Glu Arg Glu Arg Gly Ile Thr Ile Asp Ile Ser Leu Trp Lys Phe
 65 70 75 80
 Glu Thr Ser Lys Tyr Tyr Val Thr Ile Ile Asp Ala Pro Gly His Arg
 85 90 95
 Asp Phe Ile Lys Asn Met Ile Thr Gly Thr Ser Gln Ala Asp Cys Ala
 100 105 110
 Val Leu Ile Val Ala Ala Gly Val Gly Glu Phe Glu Ala Gly Ile Ser
 115 120 125
 Lys Asn Gly Gln Thr Arg Glu His Ala Leu Leu Ala Tyr Thr Leu Gly
 130 135 140
 Val Lys Gln Leu Ile Val Gly Val Asn Lys Met Asp Ser Thr Glu Pro
 145 150 155 160
 Pro Tyr Ser Gln Lys Arg Tyr Glu Glu Ile Val Lys Glu Val Ser Thr
 165 170 175
 Tyr Ile Lys Lys Ile Gly Tyr Asn Pro Asp Thr Val Ala Phe Val Pro
 180 185 190
 Ile Ser Gly Trp Asn Gly Asp Asn Met Leu Glu Pro Ser Ala Asn Met
 195 200 205
 Pro Trp Phe Lys Gly Trp Lys Val Thr Arg Lys Asp Gly Asn Ala Ser
 210 215 220
 Gly Thr Thr Leu Leu Glu Ala Val Asp Cys Ile Leu Pro Pro Thr Arg
 225 230 235 240
 Pro Thr Asp Lys Pro Leu Arg Leu Pro Leu Gln Asp Val Tyr Lys Ile
 245 250 255
 Gly Gly Ile Gly Thr Val Pro Val Gly Arg Val Glu Thr Gly Val Leu
 260 265 270
 Lys Pro Gly Met Val Val Thr Phe Ala Pro Val Asn Val Thr Thr Glu
 275 280 285
 Val Lys Ser Val Glu Met His His Glu Ala Leu Ser Glu Ala Leu Pro
 290 295 300
 Gly Asp Asn Val Gly Phe Asn Val Lys Asn Val Ser Val Lys Asp Val
 305 310 315 320
 Arg Arg Gly Asn Val Ala Gly Asp Ser Lys Asn Asp Pro Pro Met Glu
 325 330 335
 Ala Ala Gly Phe Thr Ala Gln Val Ile Ile Leu Asn His Pro Gly Gln
 340 345 350

032796-132.ST25

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Ile Ser Ala Gly Tyr Ala Pro Val Leu Asp Cys His Thr Ala His Ile
   355                               360                   365
Ala Cys Lys Phe Ala Glu Leu Lys Glu Lys Ile Asp Arg Arg Ser Gly
   370                               375                   380
Lys Lys Leu Glu Asp Gly Pro Lys Phe Leu Lys Ser Gly Asp Ala Ala
   385                               390                   395                   400
Ile Val Asp Met Val Pro Gly Lys Pro Met Cys Val Glu Ser Phe Ser
   405                               410                   415
Asp Tyr Pro Pro Leu Gly Arg Phe Ala Val Arg Asp Met Arg Gln Thr
   420                               425                   430
Val Ala Val Gly Val Ile Lys Ala Val Asp Lys Lys Ala Ala Gly Ala
   435                               440                   445
Gly Lys Val Thr Lys Ser Ala Gln Lys Ala Gln Lys Ala Lys
   450                               455                   460

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<210> 98

<211> 2328

<212> PRT

<213> Homo sapiens

<400> 98

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Lys Ser Lys Arg Gln Ala Gln Gln Met Val Gln Pro Gln Ser Pro Val
  1      5      10      15
Ala Val Ser Gln Ser Lys Pro Gly Cys Tyr Asp Asn Gly Lys His Tyr
   20      25      30
Gln Ile Asn Gln Gln Trp Glu Arg Thr Tyr Leu Gly Asn Val Leu Val
   35      40      45
Cys Thr Cys Tyr Gly Gly Ser Arg Gly Phe Asn Cys Glu Ser Lys Pro
   50      55      60
Glu Ala Glu Glu Thr Cys Phe Asp Lys Tyr Thr Gly Asn Thr Tyr Arg
   65      70      75      80
Val Gly Asp Thr Tyr Glu Arg Pro Lys Asp Ser Met Ile Trp Asp Cys
   85      90      95
Thr Cys Ile Gly Ala Gly Arg Gly Arg Ile Ser Cys Thr Ile Ala Asn
  100      105      110
Arg Cys His Glu Gly Gly Gln Ser Tyr Lys Ile Gly Asp Thr Trp Arg
  115      120      125
Arg Pro His Glu Thr Gly Gly Tyr Met Leu Glu Cys Val Cys Leu Gly
  130      135      140
Asn Gly Lys Gly Glu Trp Thr Cys Lys Pro Ile Ala Glu Lys Cys Phe
  145      150      155      160
Asp His Ala Ala Gly Thr Ser Tyr Val Val Gly Glu Thr Trp Glu Lys
  165      170      175
Pro Tyr Gln Gly Trp Met Met Val Asp Cys Thr Cys Leu Gly Glu Gly
  180      185      190
Ser Gly Arg Ile Thr Cys Thr Ser Arg Asn Arg Cys Asn Asp Gln Asp
  195      200      205
Thr Arg Thr Ser Tyr Arg Ile Gly Asp Thr Trp Ser Lys Lys Asp Asn
  210      215      220
Arg Gly Asn Leu Leu Gln Cys Ile Cys Thr Gly Asn Gly Arg Gly Glu
  225      230      235      240
Trp Lys Cys Glu Arg His Thr Ser Val Gln Thr Thr Ser Ser Gly Ser
  245      250      255
Gly Pro Phe Thr Asp Val Arg Ala Ala Val Tyr Gln Pro Gln Pro His
  260      265      270
Pro Gln Pro Pro Pro Tyr Gly His Cys Val Thr Asp Ser Gly Val Val
  275      280      285

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032796-132.ST25

Tyr Ser Val Gly Met Gln Trp Leu Lys Thr Gln Gly Asn Lys Gln Met
 290 295 300
 Leu Cys Thr Cys Leu Gly Asn Gly Val Ser Cys Gln Glu Thr Ala Val
 305 310 315 320
 Thr Gln Thr Tyr Gly Asn Leu Asn Gly Glu Pro Cys Val Leu Pro
 325 330 335
 Phe Thr Tyr Asn Gly Arg Thr Phe Tyr Ser Cys Thr Thr Glu Gly Arg
 340 345 350
 Gln Asp Gly His Leu Trp Cys Ser Thr Thr Ser Asn Tyr Glu Gln Asp
 355 360 365
 Gln Lys Tyr Ser Phe Cys Thr Asp His Thr Val Leu Val Gln Thr Gln
 370 375 380
 Gly Gly Asn Ser Asn Gly Ala Leu Cys His Phe Pro Phe Leu Tyr Asn
 385 390 395 400
 Asn His Asn Tyr Thr Asp Cys Thr Ser Glu Gly Arg Arg Asp Asn Met
 405 410 415
 Lys Trp Cys Gly Thr Thr Gln Asn Tyr Asp Ala Asp Gln Lys Phe Gly
 420 425 430
 Phe Cys Pro Met Ala Ala His Glu Glu Ile Cys Thr Thr Asn Glu Gly
 435 440 445
 Val Met Tyr Arg Ile Gly Asp Gln Trp Asp Lys Gln His Asp Met Gly
 450 455 460
 His Met Met Arg Cys Thr Cys Val Gly Asn Gly Arg Gly Glu Trp Thr
 465 470 475 480
 Cys Ile Ala Tyr Ser Gln Leu Arg Asp Gln Cys Ile Val Asp Asp Ile
 485 490 495
 Thr Tyr Asn Val Asn Asp Thr Phe His Lys Arg His Glu Glu Gly His
 500 505 510
 Met Leu Asn Cys Thr Cys Phe Gly Gln Gly Arg Gly Arg Trp Lys Cys
 515 520 525
 Asp Pro Val Asp Gln Cys Gln Asp Ser Glu Thr Gly Thr Phe Tyr Gln
 530 535 540
 Ile Gly Asp Ser Trp Glu Lys Tyr Val His Gly Val Arg Tyr Gln Cys
 545 550 555 560
 Tyr Cys Tyr Gly Arg Gly Ile Gly Glu Trp His Cys Gln Pro Leu Gln
 565 570 575
 Thr Tyr Pro Ser Ser Ser Gly Pro Val Glu Val Phe Ile Thr Glu Thr
 580 585 590
 Pro Ser Gln Pro Asn Ser His Pro Ile Gln Trp Asn Ala Pro Gln Pro
 595 600 605
 Ser His Ile Ser Lys Tyr Ile Leu Arg Trp Arg Pro Lys Asn Ser Val
 610 615 620
 Gly Arg Trp Lys Glu Ala Thr Ile Pro Gly His Leu Asn Ser Tyr Thr
 625 630 635 640
 Ile Lys Gly Leu Lys Pro Gly Val Val Tyr Glu Gly Gln Leu Ile Ser
 645 650 655
 Ile Gln Gln Tyr Gly His Gln Glu Val Thr Arg Phe Asp Phe Thr Thr
 660 665 670
 Thr Ser Thr Ser Thr Pro Val Thr Ser Asn Thr Val Thr Gly Glu Thr
 675 680 685
 Thr Pro Phe Ser Pro Leu Val Ala Thr Ser Glu Ser Val Thr Glu Ile
 690 695 700
 Thr Ala Ser Ser Phe Val Val Ser Trp Val Ser Ala Ser Asp Thr Val
 705 710 715 720
 Ser Gly Phe Arg Val Glu Tyr Glu Leu Ser Glu Glu Gly Asp Glu Pro
 725 730 735
 Gln Tyr Leu Asp Leu Pro Ser Thr Ala Thr Ser Val Asn Ile Pro Asp

032796-132.ST25

740	745	750
Leu Leu Pro Gly Arg Lys Tyr Ile Val Asn Val Tyr Gln Ile Ser Glu		
755	760	765
Asp Gly Glu Gln Ser Leu Ile Leu Ser Thr Ser Gln Thr Thr Ala Pro		
770	775	780
Asp Ala Pro Pro Asp Pro Thr Val Asp Gln Val Asp Asp Thr Ser Ile		
785	790	795
Val Val Arg Trp Ser Arg Pro Gln Ala Pro Ile Thr Gly Tyr Arg Ile		
805	810	815
Val Tyr Ser Pro Ser Val Glu Gly Ser Ser Thr Glu Leu Asn Leu Pro		
820	825	830
Glu Thr Ala Asn Ser Val Thr Leu Ser Asp Leu Gln Pro Gly Val Gln		
835	840	845
Tyr Asn Ile Thr Ile Tyr Ala Val Glu Glu Asn Gln Glu Ser Thr Pro		
850	855	860
Val Val Ile Gln Gln Glu Thr Thr Gly Thr Pro Arg Ser Asp Thr Val		
865	870	875
Pro Ser Pro Arg Asp Leu Gln Phe Val Glu Val Thr Asp Val Lys Val		
885	890	895
Thr Ile Met Trp Thr Pro Pro Glu Ser Ala Val Thr Gly Tyr Arg Val		
900	905	910
Asp Val Ile Pro Val Asn Leu Pro Gly Glu His Gly Gln Arg Leu Pro		
915	920	925
Ile Ser Arg Asn Thr Phe Ala Glu Val Thr Gly Leu Ser Pro Gly Val		
930	935	940
Thr Tyr Tyr Phe Lys Val Phe Ala Val Ser His Gly Arg Glu Ser Lys		
945	950	955
Pro Leu Thr Ala Gln Thr Thr Lys Leu Asp Ala Pro Thr Asn Leu		
965	970	975
Gln Phe Val Asn Glu Thr Asp Ser Thr Val Leu Val Arg Trp Thr Pro		
980	985	990
Pro Arg Ala Gln Ile Thr Gly Tyr Arg Leu Thr Val Gly Leu Thr Arg		
995	1000	1005
Arg Gly Gln Pro Arg Gln Tyr Asn Val Gly Pro Ser Val Ser Lys Tyr		
1010	1015	1020
Pro Leu Arg Asn Leu Gln Pro Ala Ser Glu Tyr Thr Val Ser Leu Val		
1025	1030	1035
Ala Ile Lys Gly Asn Gln Glu Ser Pro Lys Ala Thr Gly Val Phe Thr		
1045	1050	1055
Thr Leu Gln Pro Gly Ser Ser Ile Pro Pro Tyr Asn Thr Glu Val Thr		
1060	1065	1070
Glu Thr Thr Ile Val Ile Thr Trp Thr Pro Ala Pro Arg Ile Gly Phe		
1075	1080	1085
Lys Leu Gly Val Arg Pro Ser Gln Gly Gly Glu Ala Pro Arg Glu Val		
1090	1095	1100
Thr Ser Asp Ser Gly Ser Ile Val Val Ser Gly Leu Thr Pro Gly Val		
1105	1110	1115
Glu Tyr Val Tyr Thr Ile Gln Val Leu Arg Asp Gly Gln Glu Arg Asp		
1125	1130	1135
Ala Pro Ile Val Asn Lys Val Val Thr Pro Leu Ser Pro Pro Thr Asn		
1140	1145	1150
Leu His Leu Glu Ala Asn Pro Asp Thr Gly Val Leu Thr Val Ser Trp		
1155	1160	1165
Glu Arg Ser Thr Thr Pro Asp Ile Thr Gly Tyr Arg Ile Thr Thr Thr		
1170	1175	1180
Pro Thr Asn Gly Gln Gln Gly Asn Ser Leu Glu Glu Val Val His Ala		
1185	1190	1195
		1200

032796-132.ST25

Asp Gln Ser Ser Cys Thr Phe Asp Asn Leu Ser Pro Gly Leu Glu Tyr
 1205 1210 1215
 Asn Val Ser Val Tyr Thr Val Lys Asp Asp Lys Glu Ser Val Pro Ile
 1220 1225 1230
 Ser Asp Thr Ile Ile Pro Ala Val Pro Pro Pro Thr Asp Leu Arg Phe
 1235 1240 1245
 Thr Asn Ile Gly Pro Asp Thr Met Arg Val Thr Trp Ala Pro Pro Pro
 1250 1255 1260
 Ser Ile Asp Leu Thr Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn
 1265 1270 1275 1280
 Glu Glu Asp Val Ala Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val
 1285 1290 1295
 Val Leu Thr Asn Leu Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser
 1300 1305 1310
 Ser Val Tyr Glu Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys
 1315 1320 1325
 Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala
 1330 1335 1340
 Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly
 1345 1350 1355 1360
 Tyr Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu
 1365 1370 1375
 Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
 1380 1385 1390
 Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg Glu
 1395 1400 1405
 Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp Val Pro
 1410 1415 1420
 Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu Leu Ile Ser
 1425 1430 1435 1440
 Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg Ile Thr Tyr Gly
 1445 1450 1455
 Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe Thr Val Pro Gly Ser
 1460 1465 1470
 Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys Pro Gly Val Asp Tyr Thr
 1475 1480 1485
 Ile Thr Val Tyr Ala Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser
 1490 1495 1500
 Lys Pro Ile Ser Ile Asn Tyr Arg Thr Glu Ile Asp Lys Pro Ser Gln
 1505 1510 1515 1520
 Met Gln Val Thr Asp Val Gln Asp Asn Ser Ile Ser Val Lys Trp Leu
 1525 1530 1535
 Pro Ser Ser Ser Pro Val Thr Gly Tyr Arg Val Thr Thr Thr Pro Lys
 1540 1545 1550
 Asn Gly Pro Gly Pro Thr Lys Thr Lys Thr Ala Gly Pro Asp Gln Thr
 1555 1560 1565
 Glu Met Thr Ile Glu Gly Leu Gln Pro Thr Val Glu Tyr Val Val Ser
 1570 1575 1580
 Val Tyr Ala Gln Asn Pro Ser Gly Glu Ser Gln Pro Leu Val Gln Thr
 1585 1590 1595 1600
 Ala Val Thr Asn Ile Asp Arg Pro Lys Gly Leu Ala Phe Thr Asp Val
 1605 1610 1615
 Asp Val Asp Ser Ile Lys Ile Ala Trp Glu Ser Pro Gln Gly Gln Val
 1620 1625 1630
 Ser Arg Tyr Arg Val Thr Tyr Ser Ser Pro Glu Asp Gly Ile His Glu
 1635 1640 1645
 Leu Phe Pro Ala Pro Asp Gly Glu Glu Asp Thr Ala Glu Leu Gln Gly

032796-132.ST25

1650	1655	1660
Leu Arg Pro Gly Ser Glu Tyr Thr Val Ser Val Val Ala Leu His Asp		
1665	1670	1675
Asp Met Glu Ser Gln Pro Leu Ile Gly Thr Gln Ser Thr Ala Ile Pro		1680
1685	1690	1695
Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr Pro Thr Ser Leu Ser		
1700	1705	1710
Ala Gln Trp Thr Pro Pro Asn Val Gln Leu Thr Gly Tyr Arg Val Arg		
1715	1720	1725
Val Thr Pro Lys Glu Lys Thr Gly Pro Met Lys Glu Ile Asn Leu Ala		
1730	1735	1740
Pro Asp Ser Ser Ser Val Val Val Ser Gly Leu Met Val Ala Thr Lys		
1745	1750	1755
Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr Ser Arg Pro		1760
1765	1770	1775
Ala Gln Gly Val Val Thr Thr Leu Glu Asn Val Ser Pro Pro Arg Arg		
1780	1785	1790
Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp Arg		
1795	1800	1805
Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala Val Pro Ala		
1810	1815	1820
Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys Pro Asp Val Arg Ser		
1825	1830	1835
Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr Asp Tyr Lys Ile Tyr Leu		1840
1845	1850	1855
Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser Pro Val Val Ile Asp Ala		
1860	1865	1870
Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr		
1875	1880	1885
Pro Asn Ser Leu Leu Val Ser Trp Gln Pro Pro Arg Ala Arg Ile Thr		
1890	1895	1900
Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly Ser Pro Pro Arg Glu Val		
1905	1910	1915
Val Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile Thr Gly Leu		
1925	1930	1935
Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn		
1940	1945	1950
Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys Lys Thr Asp Glu Leu Pro		
1955	1960	1965
Gln Leu Val Thr Leu Pro His Pro Asn Leu His Gly Pro Glu Ile Leu		
1970	1975	1980
Asp Val Pro Ser Thr Val Gln Lys Thr Pro Phe Val Thr His Pro Gly		
1985	1990	1995
Tyr Asp Thr Gly Asn Gly Ile Gln Leu Pro Gly Thr Ser Gly Gln Gln		2000
2005	2010	2015
Pro Ser Val Gly Gln Gln Met Ile Phe Glu Glu His Gly Phe Arg Arg		
2020	2025	2030
Thr Thr Pro Pro Thr Thr Ala Thr Pro Ile Arg His Arg Pro Arg Pro		
2035	2040	2045
Tyr Pro Pro Asn Val Gly Gln Glu Ala Leu Ser Gln Thr Thr Ile Ser		
2050	2055	2060
Trp Ala Pro Phe Gln Asp Thr Ser Glu Tyr Ile Ile Ser Cys His Pro		
2065	2070	2075
Val Gly Thr Asp Glu Glu Pro Leu Gln Phe Arg Val Pro Gly Thr Ser		2080
2085	2090	2095
Thr Ser Ala Thr Leu Thr Gly Leu Thr Arg Gly Ala Thr Tyr Asn Ile		
2100	2105	2110

032796-132.ST25

Ile Val Glu Ala Leu Lys Asp Gln Gln Arg His Lys Val Arg Glu Glu
 2115 2120 2125
 Val Val Thr Val Gly Asn Ser Val Asn Glu Gly Leu Asn Gln Pro Thr
 2130 2135 2140
 Asp Asp Ser Cys Phe Asp Pro Tyr Thr Val Ser His Tyr Ala Val Gly
 2145 2150 2155 2160
 Asp Glu Trp Glu Arg Met Ser Glu Ser Gly Phe Lys Leu Leu Cys Gln
 2165 2170 2175
 Cys Leu Gly Phe Gly Ser Gly His Phe Arg Cys Asp Ser Ser Arg Trp
 2180 2185 2190
 Cys His Asp Asn Gly Val Asn Tyr Lys Ile Gly Glu Lys Trp Asp Arg
 2195 2200 2205
 Gln Gly Glu Asn Gly Gln Met Met Ser Cys Thr Cys Leu Gly Asn Gly
 2210 2215 2220
 Lys Gly Glu Phe Lys Cys Asp Pro His Glu Ala Thr Cys Tyr Asp Asp
 2225 2230 2235 2240
 Gly Lys Thr Tyr His Val Gly Glu Gln Trp Gln Lys Glu Tyr Leu Gly
 2245 2250 2255
 Ala Ile Cys Ser Cys Thr Cys Phe Gly Gly Gln Arg Gly Trp Arg Cys
 2260 2265 2270
 Asp Asn Cys Arg Arg Pro Gly Gly Glu Pro Ser Pro Glu Gly Thr Thr
 2275 2280 2285
 Gly Gln Ser Tyr Asn Gln Tyr Ser Gln Arg Tyr His Gln Arg Thr Asn
 2290 2295 2300
 Thr Asn Val Asn Cys Pro Ile Glu Cys Phe Met Pro Leu Asp Val Gln
 2305 2310 2315 2320
 Ala Asp Arg Glu Asp Ser Arg Glu
 2325

<210> 99

<211> 188

<212> PRT

<213> Homo sapiens

<400> 99

His Gln Thr His Lys Glu Gly Gly Ser Thr His Ala Ser Ala Asp Ala
 1 5 10 15
 Trp Glu Ile Ile Glu Leu Glu Thr Glu Ile Glu Lys Phe Lys Ala Glu
 20 25 30
 Asn Ala Ser Leu Ala Lys Leu Arg Ile Glu Arg Glu Ser Ala Leu Glu
 35 40 45
 Lys Leu Arg Lys Glu Ile Ala Asp Phe Glu Gln Gln Lys Ala Lys Glu
 50 55 60
 Leu Ala Arg Ile Glu Glu Phe Lys Lys Glu Glu Met Arg Lys Leu Gln
 65 70 75 80
 Lys Glu Arg Lys Val Phe Glu Lys Tyr Thr Thr Ala Ala Arg Thr Phe
 85 90 95
 Pro Asp Lys Lys Glu Arg Glu Glu Ile Gln Thr Leu Lys Gln Gln Ile
 100 105 110
 Ala Asp Leu Arg Glu Asp Leu Lys Arg Lys Glu Thr Lys Trp Ser Ser
 115 120 125
 Thr His Ser Arg Leu Arg Ser Gln Ile Gln Met Leu Val Arg Glu Asn
 130 135 140
 Thr Asp Leu Arg Glu Glu Ile Lys Val Met Glu Arg Phe Arg Leu Asp
 145 150 155 160
 Ala Trp Lys Arg Ala Glu Ala Ile Glu Ser Ser Leu Glu Val Glu Lys
 165 170 175

032796-132.ST25

Lys Asp Lys Leu Ala Asn Thr Ser Val Arg Phe Gln
 180 185

<210> 100
 <211> 284
 <212> PRT
 <213> Homo sapiens

<400> 100
 Met Glu Pro Gly Asn Tyr Ala Thr Leu Asp Gly Ala Lys Asp Ile Glu
 1 5 10 15
 Gly Leu Leu Gly Ala Gly Gly Gly Arg Asn Leu Val Ala His Ser Pro
 20 25 30
 Leu Thr Ser His Pro Ala Ala Pro Thr Leu Met Pro Ala Val Asn Tyr
 35 40 45
 Ala Pro Leu Asp Leu Pro Gly Ser Ala Glu Pro Pro Lys Gln Cys His
 50 55 60
 Pro Cys Pro Gly Val Pro Gln Gly Thr Ser Pro Ala Pro Val Pro Tyr
 65 70 75 80
 Gly Tyr Phe Gly Gly Tyr Tyr Ser Cys Arg Val Ser Arg Ser Ser
 85 90 95
 Leu Lys Pro Cys Ala Gln Ala Ala Thr Leu Ala Ala Tyr Pro Ala Glu
 100 105 110
 Thr Pro Thr Ala Gly Glu Glu Tyr Pro Ser Arg Pro Thr Glu Phe Ala
 115 120 125
 Phe Tyr Pro Gly Tyr Pro Gly Thr Tyr His Ala Met Ala Ser Tyr Leu
 130 135 140
 Asp Val Ser Val Val Gln Thr Leu Gly Ala Pro Gly Glu Pro Arg His
 145 150 155 160
 Asp Ser Leu Leu Pro Val Asp Ser Tyr Gln Ser Trp Ala Leu Ala Gly
 165 170 175
 Gly Trp Asn Ser Gln Met Cys Cys Gln Gly Glu Gln Asn Pro Pro Gly
 180 185 190
 Pro Phe Trp Lys Ala Ala Phe Ala Asp Ser Ser Gly Gln His Pro Pro
 195 200 205
 Asp Ala Cys Ala Phe Arg Arg Gly Arg Lys Lys Arg Ile Pro Tyr Ser
 210 215 220
 Lys Gly Gln Leu Arg Glu Leu Glu Arg Glu Tyr Ala Ala Asn Lys Phe
 225 230 235 240
 Ile Thr Lys Asp Lys Arg Arg Lys Ile Ser Ala Ala Thr Ser Leu Ser
 245 250 255
 Glu Arg Gln Ile Thr Ile Trp Phe Gln Asn Arg Arg Val Lys Glu Lys
 260 265 270
 Lys Val Leu Ala Lys Val Lys Asn Ser Ala Thr Pro
 275 280

<210> 101
 <211> 676
 <212> PRT
 <213> Homo sapiens

<400> 101
 Met Asp Lys Tyr Asp Asp Leu Gly Leu Glu Ala Ser Lys Phe Ile Glu
 1 5 10 15
 Asp Leu Asn Met Tyr Glu Ala Ser Lys Asp Gly Leu Phe Arg Val Asp
 20 25 30
 Lys Gly Ala Gly Asn Asn Pro Glu Phe Glu Glu Thr Arg Arg Val Phe

032796-132.ST25

35	40	45
Ala Thr Lys Met Ala Lys Ile His Leu Gln Gln Gln Gln Gln Leu		
50	55	60
Leu Gln Glu Glu Thr Leu Pro Arg Gly Ser Arg Gly Pro Val Asn Gly		
65	70	75
Gly Gly Arg Leu Gly Pro Gln Ala Arg Trp Glu Val Val Gly Ser Lys		
85	90	95
Leu Thr Val Asp Gly Ala Ala Lys Pro Pro Leu Ala Ala Ser Thr Gly		
100	105	110
Ala Pro Gly Ala Val Thr Thr Leu Ala Ala Gly Gln Pro Pro Tyr Pro		
115	120	125
Pro Gln Glu Gln Arg Ser Arg Pro Tyr Leu His Gly Thr Arg His Gly		
130	135	140
Ser Gln Asp Cys Gly Ser Arg Glu Ser Leu Ala Thr Ser Glu Met Ser		
145	150	155
Ala Phe His Gln Pro Gly Pro Cys Glu Asp Pro Ser Cys Leu Thr His		
165	170	175
Gly Asp Tyr Tyr Asp Asn Leu Ser Leu Ala Ser Pro Lys Trp Gly Asp		
180	185	190
Lys Pro Gly Val Ser Pro Ser Ile Gly Leu Ser Val Gly Ser Gly Trp		
195	200	205
Pro Ser Ser Pro Gly Ser Asp Pro Pro Leu Pro Lys Pro Cys Gly Asp		
210	215	220
His Pro Leu Asn His Arg Gln Leu Ser Leu Ser Ser Ser Arg Ser Ser		
225	230	235
Glu Gly Ser Leu Gly Gly Gln Asn Ser Gly Ile Gly Gly Arg Ser Ser		
245	250	255
Glu Lys Pro Thr Gly Leu Trp Ser Thr Ala Ser Ser Gln Arg Val Ser		
260	265	270
Pro Gly Leu Pro Ser Pro Asn Leu Glu Asn Gly Ala Pro Ala Val Gly		
275	280	285
Pro Val Gln Pro Arg Thr Pro Ser Val Ser Ala Pro Leu Ala Leu Ser		
290	295	300
Cys Pro Arg Gln Gly Gly Leu Pro Arg Ser Asn Ser Gly Leu Gly Gly		
305	310	315
Glu Val Ser Gly Val Met Ser Lys Pro Asn Val Asp Pro Gln Pro Trp		
325	330	335
Phe Gln Asp Gly Pro Lys Ser Tyr Leu Ser Ser Ser Ala Pro Ser Ser		
340	345	350
Ser Pro Ala Gly Leu Asp Gly Ser Gln Gln Gly Ala Val Pro Gly Leu		
355	360	365
Gly Pro Lys Pro Gly Cys Thr Asp Leu Gly Thr Gly Pro Lys Leu Ser		
370	375	380
Pro Thr Ser Leu Val His Pro Val Met Ser Thr Leu Pro Glu Leu Ser		
385	390	395
Cys Lys Glu Gly Pro Leu Gly Trp Ser Ser Asp Gly Ser Leu Gly Ser		
405	410	415
Val Leu Leu Asp Ser Pro Ser Ser Pro Arg Val Arg Leu Pro Cys Gln		
420	425	430
Pro Leu Val Pro Gly Pro Glu Leu Arg Pro Ser Ala Ala Glu Leu Lys		
435	440	445
Leu Glu Ala Leu Thr Gln Arg Leu Glu Arg Glu Met Asp Ala His Pro		
450	455	460
Lys Ala Asp Tyr Phe Gly Ala Cys Val Lys Cys Ser Lys Gly Val Phe		
465	470	475
Gly Ala Gly Gln Ala Cys Gln Ala Met Gly Asn Leu Tyr His Asp Thr		
485	490	495

032796-132.ST25

Cys Phe Thr Cys Ala Ala Cys Ser Arg Lys Leu Arg Gly Lys Ala Phe
 500 505 510
 Tyr Phe Val Asn Gly Lys Val Phe Cys Glu Glu Asp Phe Leu Tyr Ser
 515 520 525
 Gly Phe Gln Gln Ser Ala Asp Arg Cys Phe Leu Cys Gly His Leu Ile
 530 535 540
 Met Asp Met Ile Leu Gln Ala Leu Gly Lys Ser Tyr His Pro Gly Cys
 545 550 555 560
 Phe Arg Cys Val Ile Cys Asn Glu Cys Leu Asp Gly Val Pro Phe Thr
 565 570 575
 Val Asp Ser Glu Asn Lys Ile Tyr Cys Val Arg Asp Tyr His Lys Val
 580 585 590
 Leu Ala Pro Lys Cys Ala Ala Cys Gly Leu Pro Ile Leu Pro Pro Glu
 595 600 605
 Gly Ser Asp Glu Thr Ile Arg Val Val Ser Met Asp Arg Asp Tyr His
 610 615 620
 Val Glu Cys Tyr His Cys Glu Asp Cys Gly Leu Glu Leu Asn Asp Glu
 625 630 635 640
 Asp Gly His Arg Cys Tyr Pro Leu Glu Asp His Leu Phe Cys His Ser
 645 650 655
 Cys His Val Lys Arg Leu Glu Lys Arg Pro Ser Ser Thr Ala Leu His
 660 665 670
 Gln His His Phe
 675

<210> 102

<211> 296

<212> PRT

<213> Homo sapiens

<400> 102

Ser Thr Gly Ser Glu Phe Pro Leu Cys Thr Lys Ala Ser Pro Cys Ser
 1 5 10 15
 Ala Ala Arg Ala Gly Gly Arg Ala Leu Gly Trp Arg Leu Gln Gln Gln
 20 25 30
 Arg Glu Thr Arg Gly Asn Pro Gly Asn Pro Gly Leu Gly Val Ala Ala
 35 40 45
 Thr Met Thr Gly Ser Asn Met Ser Asp Ala Leu Ala Asn Ala Val Cys
 50 55 60
 Gln Arg Cys Gln Ala Arg Phe Ser Pro Ala Glu Arg Ile Val Asn Ser
 65 70 75 80
 Asn Gly Glu Leu Tyr His Glu His Cys Phe Val Cys Ala Gln Cys Phe
 85 90 95
 Arg Pro Phe Pro Glu Gly Leu Phe Tyr Glu Phe Glu Gly Arg Lys Tyr
 100 105 110
 Cys Glu His Asp Phe Gln Met Leu Phe Ala Pro Cys Cys Gly Ser Cys
 115 120 125
 Gly Glu Phe Ile Ile Gly Arg Val Ile Lys Ala Met Asn Asn Asn Trp
 130 135 140
 His Pro Gly Cys Phe Arg Cys Glu Leu Cys Asp Val Glu Leu Ala Asp
 145 150 155 160
 Leu Gly Phe Val Lys Asn Ala Gly Arg His Leu Cys Arg Pro Cys His
 165 170 175
 Asn Arg Glu Lys Ala Lys Gly Leu Gly Lys Tyr Ile Cys Gln Arg Cys
 180 185 190
 His Leu Val Ile Asp Glu Gln Pro Leu Met Phe Arg Ser Asp Ala Tyr
 195 200 205

032796-132.ST25

His Pro Asp His Phe Asn Cys Thr His Cys Gly Lys Glu Leu Thr Ala
 210 215 220
 Glu Ala Arg Glu Leu Lys Gly Glu Leu Tyr Cys Leu Pro Cys His Asp
 225 230 235 240
 Lys Met Gly Val Pro Ile Cys Gly Ala Cys Arg Arg Pro Ile Glu Gly
 245 250 255
 Arg Val Val Asn Ala Leu Gly Lys Gln Trp His Val Glu His Phe Val
 260 265 270
 Cys Ala Lys Cys Glu Lys Pro Phe Leu Gly His Arg His Tyr Glu Lys
 275 280 285
 Lys Gly Leu Ala Tyr Cys Glu Leu
 290 295

<210> 103

<211> 500

<212> PRT

<213> Homo sapiens

<400> 103

Met Gly Ile Gly Leu Ser Ala Gln Gly Val Asn Met Asn Arg Leu Pro
 1 5 10 15
 Gly Trp Asp Lys His Ser Tyr Gly Tyr His Gly Asp Asp Gly His Ser
 20 25 30
 Phe Cys Ser Ser Gly Thr Gly Gln Pro Tyr Gly Pro Thr Phe Thr Thr
 35 40 45
 Gly Asp Val Ile Gly Cys Cys Val Asn Leu Ile Asn Asn Thr Cys Phe
 50 55 60
 Tyr Thr Lys Asn Gly His Ser Leu Gly Ile Ala Phe Thr Asp Leu Pro
 65 70 75 80
 Pro Asn Leu Tyr Pro Thr Val Gly Leu Gln Thr Pro Gly Glu Val Val
 85 90 95
 Asp Ala Asn Phe Gly Gln His Pro Phe Val Phe Asp Ile Glu Asp Tyr
 100 105 110
 Met Arg Glu Trp Arg Thr Lys Ile Gln Ala Gln Ile Asp Arg Phe Pro
 115 120 125
 Ile Gly Asp Arg Glu Gly Glu Trp Gln Thr Met Ile Gln Lys Met Val
 130 135 140
 Ser Ser Tyr Leu Val His His Gly Tyr Cys Ala Thr Ala Glu Ala Phe
 145 150 155 160
 Ala Arg Ser Thr Asp Gln Thr Val Leu Glu Glu Leu Ala Ser Ile Lys
 165 170 175
 Asn Arg Gln Arg Ile Gln Lys Leu Val Leu Ala Gly Arg Met Gly Glu
 180 185 190
 Ala Ile Glu Thr Thr Gln Gln Leu Tyr Pro Ser Leu Leu Glu Arg Asn
 195 200 205
 Pro Asn Leu Leu Phe Thr Leu Lys Val Arg Gln Phe Ile Glu Met Val
 210 215 220
 Asn Gly Thr Asp Ser Glu Val Arg Cys Leu Gly Gly Arg Ser Pro Lys
 225 230 235 240
 Ser Gln Asp Ser Tyr Pro Val Ser Pro Arg Pro Phe Ser Ser Pro Ser
 245 250 255
 Met Ser Pro Ser His Gly Met Asn Ile His Asn Leu Ala Ser Gly Lys
 260 265 270
 Gly Ser Thr Ala His Phe Ser Gly Phe Glu Ser Cys Ser Asn Gly Val
 275 280 285
 Ile Ser Asn Lys Ala His Gln Ser Tyr Cys His Ser Asn Lys His Gln
 290 295 300

032796-132.ST25

Ser Ser Asn Leu Asn Val Pro Glu Leu Asn Ser Ile Asn Met Ser Arg
 305 310 315 320
 Ser Gln Gln Val Asn Asn Phe Thr Ser Asn Asp Val Asp Met Glu Thr
 325 330 335
 Asp His Tyr Ser Asn Gly Val Gly Glu Thr Ser Ser Asn Gly Phe Leu
 340 345 350
 Asn Gly Ser Ser Lys His Asp His Glu Met Glu Asp Cys Asp Thr Glu
 355 360 365
 Met Glu Val Asp Ser Ser Gln Leu Arg Arg Gln Leu Cys Gly Gly Ser
 370 375 380
 Gln Ala Ala Ile Glu Arg Met Ile His Phe Gly Arg Glu Leu Gln Ala
 385 390 395 400
 Met Ser Glu Gln Leu Arg Arg Asp Cys Gly Lys Asn Thr Ala Asn Lys
 405 410 415
 Lys Met Leu Lys Asp Ala Phe Ser Leu Leu Ala Tyr Ser Asp Pro Trp
 420 425 430
 Asn Ser Pro Val Gly Asn Gln Leu Asp Pro Ile Gln Arg Glu Pro Val
 435 440 445
 Cys Ser Ala Leu Asn Ser Ala Ile Leu Glu Thr His Asn Leu Pro Lys
 450 455 460
 Gln Pro Pro Leu Ala Leu Ala Met Gly Gln Ala Thr Gln Cys Leu Gly
 465 470 475 480
 Leu Met Ala Arg Ser Gly Ile Gly Ser Cys Ala Phe Ala Thr Val Glu
 485 490 495
 Asp Tyr Leu His
 500

<210> 104

<211> 387

<212> PRT

<213> Homo sapiens

<400> 104

Met Ala Thr Ser Gly Val Leu Pro Gly Gly Gly Phe Val Ala Ser Ala
 1 5 10 15
 Ala Ala Val Ala Gly Pro Glu Met Gln Thr Gly Arg Asn Asn Phe Val
 20 25 30
 Ile Arg Arg Asn Pro Ala Asp Pro Gln Arg Ile Pro Ser Asn Pro Ser
 35 40 45
 His Arg Ile Gln Cys Ala Ala Gly Tyr Glu Gln Ser Glu His Asn Val
 50 55 60
 Cys Gln Asp Ile Asp Glu Cys Thr Ala Gly Thr His Asn Cys Arg Ala
 65 70 75 80
 Asp Gln Val Cys Ile Asn Leu Arg Gly Ser Phe Ala Cys Gln Cys Pro
 85 90 95
 Pro Gly Tyr Gln Lys Arg Gly Glu Gln Cys Val Asp Ile Asp Glu Cys
 100 105 110
 Thr Ile Pro Pro Tyr Cys His Gln Arg Cys Val Asn Thr Pro Gly Ser
 115 120 125
 Phe Tyr Cys Gln Cys Ser Pro Gly Phe Gln Leu Ala Ala Asn Asn Tyr
 130 135 140
 Thr Cys Val Asp Ile Asn Glu Cys Asp Ala Ser Asn Gln Cys Ala Gln
 145 150 155 160
 Gln Cys Tyr Asn Ile Leu Gly Ser Phe Ile Cys Gln Cys Asn Gln Gly
 165 170 175
 Tyr Glu Leu Ser Ser Asp Arg Leu Asn Cys Glu Asp Ile Asp Glu Cys
 180 185 190

032796-132.ST25

Arg Thr Ser Ser Tyr Leu Cys Gln Tyr Gln Cys Val Asn Glu Pro Gly
 195 200 205
 Lys Phe Ser Cys Met Cys Pro Gln Gly Tyr Gln Val Val Arg Ser Arg
 210 215 220
 Thr Cys Gln Asp Ile Asn Glu Cys Glu Thr Thr Asn Glu Cys Arg Glu
 225 230 235 240
 Asp Glu Met Cys Trp Asn Tyr His Gly Gly Phe Arg Cys Tyr Pro Arg
 245 250 255
 Asn Pro Cys Gln Asp Pro Tyr Ile Leu Thr Pro Glu Asn Arg Cys Val
 260 265 270
 Cys Pro Val Ser Asn Ala Met Cys Arg Glu Leu Pro Gln Ser Ile Val
 275 280 285
 Tyr Lys Tyr Met Ser Ile Arg Ser Asp Arg Ser Val Pro Ser Asp Ile
 290 295 300
 Phe Gln Ile Gln Ala Thr Thr Ile Tyr Ala Asn Thr Ile Asn Thr Phe
 305 310 315 320
 Arg Ile Lys Ser Gly Asn Glu Asn Gly Glu Phe Tyr Leu Arg Gln Thr
 325 330 335
 Ser Pro Val Ser Ala Met Leu Val Leu Val Lys Ser Leu Ser Gly Pro
 340 345 350
 Arg Glu His Ile Val Asp Leu Glu Met Leu Thr Val Ser Ser Ile Gly
 355 360 365
 Thr Phe Arg Thr Ser Ser Val Leu Arg Leu Thr Ile Ile Val Gly Pro
 370 375 380
 Phe Ser Phe
 385

<210> 105

<211> 531

<212> PRT

<213> Homo sapiens

<400> 105

Met Ser Lys Pro His Ser Glu Ala Gly Thr Ala Phe Ile Gln Thr Gln
 1 5 10 15
 Gln Leu His Ala Ala Met Ala Asp Thr Phe Leu Glu His Met Cys Arg
 20 25 30
 Leu Asp Ile Asp Ser Pro Pro Ile Thr Ala Arg Asn Thr Gly Ile Ile
 35 40 45
 Cys Thr Ile Gly Pro Ala Ser Arg Ser Val Glu Thr Leu Lys Glu Met
 50 55 60
 Ile Lys Ser Gly Met Asn Val Ala Arg Leu Asn Phe Ser His Gly Thr
 65 70 75 80
 His Glu Tyr His Ala Glu Thr Ile Lys Asn Val Arg Thr Ala Thr Glu
 85 90 95
 Ser Phe Ala Ser Asp Pro Tyr Leu Tyr Arg Pro Val Ala Val Ala Leu
 100 105 110
 Asp Thr Lys Gly Pro Glu Ile Arg Thr Gly Leu Ile Lys Gly Ser Gly
 115 120 125
 Thr Ala Glu Leu Glu Leu Lys Lys Gly Ala Thr Leu Lys Ile Thr Leu
 130 135 140
 Asp Asn Ala Tyr Met Glu Lys Cys Asp Glu Asn Ile Leu Trp Leu Asp
 145 150 155 160
 Tyr Lys Asn Ile Cys Lys Val Val Glu Val Gly Ser Lys Ile Tyr Val
 165 170 175
 Asp Asp Gly Leu Ile Ser Leu Gln Val Lys Gln Lys Gly Ala Asp Phe
 180 185 190

032796-132.ST25

Leu Val Thr Glu Val Glu Asn Gly Gly Ser Leu Gly Ser Lys Lys Gly
 195 200 205
 Val Asn Leu Pro Gly Ala Ala Val Asp Leu Pro Ala Val Ser Glu Lys
 210 215 220
 Asp Ile Gln Asp Leu Lys Phe Gly Val Glu Gln Asp Val Asp Met Val
 225 230 235 240
 Phe Ala Ser Phe Ile Arg Lys Ala Ser Asp Val His Glu Val Arg Lys
 245 250 255
 Val Leu Gly Glu Lys Gly Lys Asn Ile Lys Ile Ile Ser Lys Ile Glu
 260 265 270
 Asn His Glu Gly Val Arg Arg Phe Asp Glu Ile Leu Glu Ala Ser Asp
 275 280 285
 Gly Ile Met Val Ala Arg Gly Asp Leu Gly Ile Glu Ile Pro Ala Glu
 290 295 300
 Lys Val Phe Leu Ala Gln Lys Met Met Ile Gly Arg Cys Asn Arg Ala
 305 310 315 320
 Gly Lys Pro Val Ile Cys Ala Thr Gln Met Leu Glu Ser Met Ile Lys
 325 330 335
 Lys Pro Arg Pro Thr Arg Ala Glu Gly Ser Asp Val Ala Asn Ala Val
 340 345 350
 Leu Asp Gly Ala Asp Cys Ile Met Leu Ser Gly Glu Thr Ala Lys Gly
 355 360 365
 Asp Tyr Pro Leu Glu Ala Val Arg Met Gln His Leu Ile Ala Arg Glu
 370 375 380
 Ala Glu Ala Ala Ile Tyr His Leu Gln Leu Phe Glu Glu Leu Arg Arg
 385 390 395 400
 Leu Ala Pro Ile Thr Ser Asp Pro Thr Glu Ala Thr Ala Val Gly Ala
 405 410 415
 Val Glu Ala Ser Phe Lys Cys Cys Ser Gly Ala Ile Ile Val Leu Thr
 420 425 430
 Lys Ser Gly Arg Ser Ala His Gln Val Ala Arg Tyr Arg Pro Arg Ala
 435 440 445
 Pro Ile Ile Ala Val Thr Arg Asn Pro Gln Thr Ala Arg Gln Ala His
 450 455 460
 Leu Tyr Arg Gly Ile Phe Pro Val Leu Cys Lys Asp Pro Val Gln Glu
 465 470 475 480
 Ala Trp Ala Glu Asp Val Asp Leu Arg Val Asn Phe Ala Met Asn Val
 485 490 495
 Gly Lys Ala Arg Gly Phe Phe Lys Lys Gly Asp Val Val Ile Val Leu
 500 505 510
 Thr Gly Trp Arg Pro Gly Ser Gly Phe Thr Asn Thr Met Arg Val Val
 515 520 525
 Pro Val Pro
 530

<210> 106

<211> 480

<212> PRT

<213> Homo sapiens

<400> 106

Met Ala Ala Arg Cys Ser Thr Arg Trp Leu Leu Val Val Val Gly Thr
 1 5 10 15
 Pro Arg Leu Pro Ala Ile Ser Gly Arg Gly Ala Arg Pro Pro Arg Glu
 20 25 30
 Gly Val Val Gly Ala Trp Leu Ser Arg Lys Leu Ser Val Pro Ala Phe
 35 40 45

032796-132.ST25

Ala Ser Ser Leu Thr Ser Cys Gly Pro Arg Ala Leu Leu Thr Leu Arg
 50 55 60
 Pro Gly Val Ser Leu Thr Gly Thr Lys His Asn Pro Phe Ile Cys Thr
 65 70 75 80
 Ala Ser Phe His Thr Ser Ala Pro Leu Ala Lys Glu Asp Tyr Tyr Gln
 85 90 95
 Ile Leu Gly Val Pro Arg Asn Ala Ser Gln Lys Glu Ile Lys Lys Ala
 100 105 110
 Tyr Tyr Gln Leu Ala Lys Lys Tyr His Pro Asp Thr Asn Lys Asp Asp
 115 120 125
 Pro Lys Ala Lys Glu Lys Phe Ser Gln Leu Ala Glu Ala Tyr Glu Val
 130 135 140
 Leu Ser Asp Glu Val Lys Arg Lys Gln Tyr Asp Ala Tyr Gly Ser Ala
 145 150 155 160
 Gly Phe Asp Pro Gly Ala Ser Gly Ser Gln His Ser Tyr Trp Lys Gly
 165 170 175
 Gly Pro Thr Val Asp Pro Glu Glu Leu Phe Arg Lys Ile Phe Gly Glu
 180 185 190
 Phe Ser Ser Ser Ser Phe Gly Asp Phe Gln Thr Val Phe Asp Gln Pro
 195 200 205
 Gln Glu Tyr Phe Met Glu Leu Thr Phe Asn Gln Ala Ala Lys Gly Val
 210 215 220
 Asn Lys Glu Phe Thr Val Asn Ile Met Asp Thr Cys Glu Arg Cys Asn
 225 230 235 240
 Gly Lys Gly Asn Glu Pro Gly Thr Lys Val Gln His Cys His Tyr Cys
 245 250 255
 Gly Gly Ser Gly Met Glu Thr Ile Asn Thr Gly Pro Phe Val Met Arg
 260 265 270
 Ser Thr Cys Arg Arg Cys Gly Gly Arg Gly Ser Ile Ile Ile Ser Pro
 275 280 285
 Cys Val Val Cys Arg Gly Ala Gly Gln Ala Lys Gln Lys Lys Arg Val
 290 295 300
 Met Ile Pro Val Pro Ala Gly Val Glu Asp Gly Gln Thr Val Arg Met
 305 310 315 320
 Pro Val Gly Lys Arg Glu Ile Phe Ile Thr Phe Arg Val Gln Lys Ser
 325 330 335
 Pro Val Phe Arg Arg Asp Gly Ala Asp Ile His Ser Asp Leu Phe Ile
 340 345 350
 Ser Ile Ala Gln Ala Leu Leu Gly Gly Thr Ala Arg Ala Gln Gly Leu
 355 360 365
 Tyr Glu Thr Ile Asn Val Thr Ile Pro Pro Gly Thr Gln Thr Asp Gln
 370 375 380
 Lys Ile Arg Met Gly Gly Lys Gly Ile Pro Arg Ile Asn Ser Tyr Gly
 385 390 395 400
 Tyr Gly Asp His Tyr Ile His Ile Lys Ile Arg Val Pro Lys Arg Leu
 405 410 415
 Thr Ser Arg Gln Gln Ser Leu Ile Leu Ser Tyr Ala Glu Asp Glu Thr
 420 425 430
 Asp Val Glu Gly Thr Val Asn Gly Val Thr Leu Thr Ser Ser Gly Gly
 435 440 445
 Ser Thr Met Asp Ser Ser Ala Gly Ser Lys Ala Arg Arg Glu Ala Gly
 450 455 460
 Glu Asp Glu Glu Gly Phe Leu Ser Lys Leu Lys Lys Met Phe Thr Ser
 465 470 475 480

<210> 107

<211> 572

032796-132.ST25

<212> PRT

<213> Homo sapiens

<400> 107

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Met Ala Ala Pro Arg Pro Ser Pro Ala Ile Ser Val Ser Val Ser Ala
1      5      10      15
Pro Ala Phe Tyr Ala Pro Gln Lys Lys Phe Gly Pro Val Val Ala Pro
20     25     30
Lys Pro Lys Val Asn Pro Phe Arg Pro Gly Asp Ser Glu Pro Pro Pro
35     40     45
Ala Pro Gly Ala Gln Arg Ala Gln Met Gly Arg Val Gly Glu Ile Pro
50     55     60
Pro Pro Pro Pro Glu Asp Phe Pro Leu Pro Pro Pro Leu Ala Gly
65     70     75     80
Asp Gly Asp Asp Ala Glu Gly Ala Leu Gly Gly Ala Phe Pro Pro Pro
85     90     95
Pro Pro Pro Ile Glu Glu Ser Phe Pro Pro Ala Pro Leu Glu Glu Glu
100    105    110
Ile Phe Pro Ser Pro Pro Pro Pro Pro Glu Glu Glu Gly Gly Pro Glu
115    120    125
Ala Pro Ile Pro Pro Pro Pro Gln Pro Arg Glu Lys Val Ser Ser Ile
130    135    140
Asp Leu Glu Ile Asp Ser Leu Ser Ser Leu Leu Asp Asp Met Thr Lys
145    150    155    160
Asn Asp Pro Phe Lys Ala Arg Val Ser Ser Gly Tyr Val Pro Pro Pro
165    170    175
Val Ala Thr Pro Phe Ser Ser Lys Ser Ser Thr Lys Pro Ala Ala Gly
180    185    190
Gly Thr Ala Pro Leu Pro Pro Trp Lys Ser Pro Ser Ser Ser Gln Pro
195    200    205
Leu Pro Gln Val Pro Ala Pro Ala Gln Ser Gln Thr Gln Phe His Val
210    215    220
Gln Pro Gln Pro Gln Pro Lys Pro Gln Val Gln Leu His Val Gln Ser
225    230    235    240
Gln Thr Gln Pro Val Ser Leu Ala Asn Thr Gln Pro Arg Gly Pro Pro
245    250    255
Ala Ser Ser Pro Ala Pro Ala Pro Lys Phe Ser Pro Val Thr Pro Lys
260    265    270
Phe Thr Pro Val Ala Ser Lys Phe Ser Pro Gly Ala Pro Gly Gly Ser
275    280    285
Gly Ser Gln Pro Asn Gln Lys Leu Gly His Pro Glu Ala Leu Ser Ala
290    295    300
Gly Thr Gly Ser Pro Gln Pro Pro Ser Phe Thr Tyr Ala Gln Gln Arg
305    310    315    320
Glu Lys Pro Arg Val Gln Glu Lys Gln His Pro Val Pro Pro Pro Ala
325    330    335
Gln Asn Gln Asn Gln Val Arg Ser Pro Gly Ala Pro Gly Pro Leu Thr
340    345    350
Leu Lys Glu Val Glu Glu Leu Glu Gln Leu Thr Gln Gln Leu Met Gln
355    360    365
Asp Met Glu His Pro Gln Arg Gln Asn Val Ala Val Asn Glu Leu Cys
370    375    380
Gly Arg Cys His Gln Pro Leu Ala Arg Ala Gln Pro Ala Val Arg Ala
385    390    395    400
Leu Gly Gln Leu Phe His Ile Ala Cys Phe Thr Cys His Gln Cys Ala
405    410    415
Gln Gln Leu Gln Gly Gln Gln Phe Tyr Ser Leu Glu Gly Ala Pro Tyr

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032796-132.ST25

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      420      425      430
Cys Glu Gly Cys Tyr Thr Asp Thr Leu Glu Lys Cys Asn Thr Cys Gly
      435      440      445
Glu Pro Ile Thr Asp Arg Met Leu Arg Ala Thr Gly Lys Ala Tyr His
      450      455      460
Pro His Cys Phe Thr Cys Val Val Cys Ala Arg Pro Leu Glu Gly Thr
465      470      475      480
Ser Phe Ile Val Asp Gln Ala Asn Arg Pro His Cys Val Pro Asp Tyr
      485      490      495
His Lys Gln Tyr Ala Pro Arg Cys Ser Val Cys Ser Glu Pro Ile Met
      500      505      510
Pro Glu Pro Gly Arg Asp Glu Thr Val Arg Val Val Ala Leu Asp Lys
      515      520      525
Asn Phe His Met Lys Cys Tyr Lys Cys Glu Asp Cys Gly Lys Pro Leu
530      535      540
Ser Ile Glu Ala Asp Asp Asn Gly Cys Phe Pro Leu Asp Gly His Val
545      550      555      560
Leu Cys Arg Lys Cys His Thr Ala Arg Ala Gln Thr
      565      570

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<210> 108

<211> 2861

<212> PRT

<213> Homo sapiens

<400> 108

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Met Lys Ala Met Asp Val Leu Pro Ile Leu Lys Glu Lys Val Ala Tyr
 1      5      10      15
Leu Ser Gly Gly Arg Asp Lys Arg Gly Gly Pro Ile Leu Thr Phe Pro
      20      25      30
Ala Arg Ser Asn His Asp Arg Ile Arg Gln Glu Asp Leu Arg Arg Leu
      35      40      45
Ile Ser Tyr Leu Ala Cys Ile Pro Ser Glu Glu Val Cys Lys Arg Gly
50      55      60
Phe Thr Val Ile Val Asp Met Arg Gly Ser Lys Trp Asp Ser Ile Lys
65      70      75      80
Pro Leu Leu Lys Ile Leu Gln Glu Ser Phe Pro Cys Cys Ile His Val
      85      90      95
Ala Leu Ile Ile Lys Pro Asp Asn Phe Trp Gln Lys Gln Arg Thr Asn
      100      105      110
Phe Gly Ser Ser Lys Phe Glu Phe Glu Thr Asn Met Val Ser Leu Glu
      115      120      125
Gly Leu Thr Lys Val Val Asp Pro Ser Gln Leu Thr Pro Glu Phe Asp
130      135      140
Gly Cys Leu Glu Tyr Asn His Glu Glu Trp Ile Glu Ile Arg Val Ala
145      150      155      160
Phe Glu Asp Tyr Ile Ser Asn Ala Thr His Met Leu Ser Arg Leu Glu
      165      170      175
Glu Leu Gln Asp Ile Leu Ala Lys Lys Glu Leu Pro Gln Asp Leu Glu
      180      185      190
Gly Ala Arg Asn Met Ile Glu Glu His Ser Gln Leu Lys Lys Lys Val
      195      200      205
Ile Lys Ala Pro Ile Glu Asp Leu Asp Leu Glu Gly Gln Lys Leu Leu
210      215      220
Gln Arg Ile Gln Ser Ser Glu Ser Phe Pro Lys Lys Asn Ser Gly Ser
225      230      235      240
Gly Asn Ala Asp Leu Gln Asn Leu Leu Pro Lys Val Ser Thr Met Leu

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															245								250								255		
Asp	Arg	Leu	His			Ser	Thr	Arg	Gln	His			Leu	His	Gln	Met	Trp			His	Val												
															260								265								270		
Arg	Lys	Leu	Lys	Leu	Asp	Gln	Cys			Phe	Gln	Leu	Arg	Leu			Phe	Glu	Gln														
															275								280								285		
Asp	Ala	Glu	Lys	Met	Phe	Asp	Trp	Ile	Thr	His	Asn	Lys	Gly	Leu	Phe																		
															290								300								305		
Leu	Asn	Ser	Tyr	Thr	Glu	Ile	Gly	Thr	Ser	His	Pro	His	Ala	Met	Glu																		
															310								315								320		
Leu	Gln	Thr	Gln	His	Asn	His	Phe	Ala	Met	Asn	Cys	Met	Asn	Val	Tyr																		
															325								330								335		
Val	Asn	Ile	Asn	Arg	Ile	Met	Ser	Val	Ala	Asn	Arg	Leu	Val	Glu	Ser																		
															340								345								350		
Gly	His	Tyr	Ala	Ser	Gln	Gln	Ile	Arg	Gln	Ile	Ala	Ser	Gln	Leu	Glu																		
															355								360								365		
Gln	Glu	Trp	Lys	Ala	Phe	Ala	Ala	Ala	Leu	Asp	Glu	Arg	Ser	Thr	Leu																		
															370								375								380		
Leu	Asp	Met	Ser	Ser	Ile	Phe	His	Gln	Lys	Ala	Glu	Lys	Tyr	Met	Ser																		
															385								390								395		
Asn	Val	Asp	Ser	Trp	Cys	Lys	Ala	Cys	Gly	Glu	Val	Asp	Leu	Pro	Ser																		
															405								410								415		
Glu	Leu	Gln	Asp	Leu	Glu	Asp	Ala	Ile	His	His	His	Gln	Gly	Ile	Tyr																		
															420								425								430		
Glu	His	Ile	Thr	Leu	Ala	Tyr	Ser	Glu	Val	Ser	Gln	Asp	Gly	Lys	Ser																		
															435								440								445		
Leu	Leu	Asp	Lys	Leu	Gln	Arg	Pro	Leu	Thr	Pro	Gly	Ser	Ser	Asp	Ser																		
															450								455								460		
Leu	Thr	Ala	Ser	Ala	Asn	Tyr	Ser	Lys	Ala	Val	His	His	Val	Leu	Asp																		
															465								470								475		
Val	Ile	His	Glu	Val	Leu	His	His	Gln	Arg	His	Val	Arg	Thr	Ile	Trp																		
															485								490								495		
Gln	His	Arg	Lys	Val	Arg	Leu	His	Gln	Arg	Leu	Gln	Leu	Cys	Val	Phe																		
															500								505								510		
Gln	Gln	Glu	Val	Gln	Gln	Val	Leu	Asp	Trp	Ile	Glu	Asn	His	Gly	Glu																		
															515								520								525		
Ala	Phe	Leu	Ser	Lys	His	Thr	Gly	Val	Gly	Lys	Ser	Leu	His	Arg	Ala																		
															530								535								540		
Arg	Ala	Leu	Gln	Lys	Arg	His	Glu	Asp	Phe	Glu	Val	Ala	Gln	Asn	Trp																		
															545								550								555		
Thr	Tyr	Thr	Asn	Ala	Asp	Lys	Leu	Leu	Glu	Ala	Ala	Glu	Gln	Leu	Ala																		
															565								570								575		
Gln	Thr	Gly	Glu	Cys	Asp	Pro	Glu	Glu	Ile	Tyr	Gln	Ala	Ala	His	Gln																		
															580								585								590		
Leu	Glu	Asp	Arg	Ile	Gln	Asp	Phe	Val	Arg	Arg	Val	Glu	Gln	Arg	Lys																		
															595								600								605		
Ile	Leu	Leu	Asp	Met	Ser	Val	Ser	Phe	His	Thr	His	Val	Lys	Glu	Leu																		

032796-132.ST25

Leu Asp Glu Ala Gln Ser Gln Met Glu Glu Leu Phe Gln Glu Arg Lys
 705 710 715 720
 Ile Lys Leu Glu Leu Phe Leu His Val Arg Ile Phe Glu Arg Asp Ala
 725 730 735
 Ile Asp Ile Ile Ser Asp Leu Glu Ser Trp Asn Asp Glu Leu Ser Gln
 740 745 750
 Gln Met Asn Asp Phe Asp Thr Glu Asp Leu Thr Ile Ala Glu Gln Arg
 755 760 765
 Leu Gln His His Ala Asp Lys Ala Leu Thr Met Asn Asn Leu Thr Phe
 770 775 780
 Asp Val Ile His Gln Gly Gln Asp Leu Leu Gln Tyr Val Asn Glu Val
 785 790 795 800
 Gln Ala Ser Gly Val Glu Leu Leu Cys Asp Arg Asp Val Asp Met Ala
 805 810 815
 Thr Arg Val Gln Asp Leu Leu Glu Phe Leu His Glu Lys Gln Gln Glu
 820 825 830
 Leu Asp Leu Ala Ala Glu Gln His Arg Lys His Leu Glu Gln Cys Val
 835 840 845
 Gln Leu Arg His Leu Gln Ala Glu Val Lys Gln Val Leu Gly Trp Ile
 850 855 860
 Arg Asn Gly Glu Ser Met Leu Asn Ala Gly Leu Ile Thr Ala Ser Ser
 865 870 875 880
 Leu Gln Glu Ala Glu Gln Leu Gln Arg Glu His Glu Gln Phe Gln His
 885 890 895
 Ala Ile Glu Lys Thr His Gln Ser Ala Leu Gln Val Gln Gln Lys Ala
 900 905 910
 Glu Ala Met Leu Gln Ala Asn His Tyr Asp Met Asp Met Ile Arg Asp
 915 920 925
 Cys Ala Glu Lys Val Ala Ser His Trp Gln Gln Leu Met Leu Lys Met
 930 935 940
 Glu Asp Arg Leu Lys Leu Val Asn Ala Ser Val Ala Phe Tyr Lys Thr
 945 950 955 960
 Ser Glu Gln Val Cys Ser Val Leu Glu Ser Leu Glu Gln Glu Tyr Lys
 965 970 975
 Arg Glu Glu Asp Trp Cys Gly Gly Ala Asp Lys Leu Gly Pro Asn Ser
 980 985 990
 Glu Thr Asp His Val Thr Pro Met Ile Ser Lys His Leu Glu Gln Lys
 995 1000 1005
 Glu Ala Phe Leu Lys Ala Cys Thr Leu Ala Arg Arg Asn Ala Asp Val
 1010 1015 1020
 Phe Leu Lys Tyr Leu His Arg Asn Ser Val Asn Met Pro Gly Met Val
 1025 1030 1035 1040
 Thr His Ile Lys Ala Pro Glu Gln Gln Val Lys Asn Ile Leu Asn Glu
 1045 1050 1055
 Leu Phe Gln Arg Glu Asn Arg Val Leu His Tyr Trp Thr Met Arg Lys
 1060 1065 1070
 Arg Arg Leu Asp Gln Cys Gln Gln Tyr Val Val Phe Glu Arg Ser Ala
 1075 1080 1085
 Lys Gln Ala Leu Glu Trp Ile His Asp Asn Gly Glu Phe Tyr Leu Ser
 1090 1095 1100
 Thr His Thr Ser Thr Gly Ser Ser Ile Gln His Thr Gln Glu Leu Leu
 1105 1110 1115 1120
 Lys Glu His Glu Glu Phe Gln Ile Thr Ala Lys Gln Thr Lys Glu Arg
 1125 1130 1135
 Val Lys Leu Leu Ile Gln Leu Ala Asp Gly Phe Cys Glu Lys Gly His
 1140 1145 1150
 Ala His Ala Ala Glu Ile Lys Lys Cys Val Thr Ala Val Asp Lys Arg

032796-132.ST25

1155	1160	1165
Tyr Arg Asp Phe Ser Leu Arg Met Glu Lys Tyr Arg Thr Ser Leu Glu		
1170	1175	1180
Lys Ala Leu Gly Ile Ser Ser Asp Ser Asn Lys Ser Ser Lys Ser Leu		
1185	1190	1195
Gln Leu Asp Ile Ile Pro Ala Ser Ile Pro Gly Ser Glu Val Lys Leu		1200
1205	1210	1215
Arg Asp Ala Ala His Glu Leu Asn Glu Glu Lys Arg Lys Ser Ala Arg		
1220	1225	1230
Arg Lys Glu Phe Ile Met Ala Glu Leu Ile Gln Thr Glu Lys Ala Tyr		
1235	1240	1245
Val Arg Asp Leu Arg Glu Cys Met Asp Thr Tyr Leu Trp Glu Met Thr		
1250	1255	1260
Ser Gly Val Glu Glu Ile Pro Pro Gly Ile Val Asn Lys Glu Leu Ile		
1265	1270	1275
Ile Phe Gly Asn Met Gln Glu Ile Tyr Glu Phe His Asn Asn Ile Phe		1280
1285	1290	1295
Leu Lys Glu Leu Glu Lys Tyr Glu Gln Leu Pro Glu Asp Val Gly His		
1300	1305	1310
Cys Phe Val Thr Trp Ala Asp Lys Phe Gln Met Tyr Val Thr Tyr Cys		
1315	1320	1325
Lys Asn Lys Pro Asp Ser Thr Gln Leu Ile Leu Glu His Ala Gly Ser		
1330	1335	1340
Tyr Phe Asp Glu Ile Gln Gln Arg His Gly Leu Ala Asn Ser Ile Ser		
1345	1350	1355
Ser Tyr Leu Ile Lys Pro Val Gln Arg Ile Thr Lys Tyr Gln Leu Leu		1360
1365	1370	1375
Leu Lys Glu Leu Leu Thr Cys Cys Glu Glu Gly Lys Gly Glu Ile Lys		
1380	1385	1390
Asp Gly Leu Glu Val Met Leu Ser Val Pro Lys Arg Ala Asn Asp Ala		
1395	1400	1405
Met His Leu Ser Met Leu Glu Gly Phe Asp Glu Asn Ile Glu Ser Gln		
1410	1415	1420
Gly Glu Leu Ile Leu Gln Glu Ser Phe Gln Val Trp Asp Pro Lys Thr		
1425	1430	1435
Leu Ile Arg Lys Gly Arg Glu Arg His Leu Phe Leu Phe Glu Met Ser		1440
1445	1450	1455
Leu Val Phe Ser Lys Glu Val Lys Asp Ser Ser Gly Arg Ser Lys Tyr		
1460	1465	1470
Leu Tyr Lys Ser Lys Leu Phe Thr Ser Glu Leu Gly Val Thr Glu His		
1475	1480	1485
Val Glu Gly Asp Pro Cys Lys Phe Ala Leu Trp Val Gly Arg Thr Pro		
1490	1495	1500
Thr Ser Asp Asn Lys Ile Val Leu Lys Ala Ser Ser Ile Glu Asn Lys		
1505	1510	1515
Gln Asp Trp Ile Lys His Ile Arg Glu Val Ile Gln Glu Arg Thr Ile		1520
1525	1530	1535
His Leu Lys Gly Ala Leu Lys Glu Pro Ile His Ile Pro Lys Thr Ala		
1540	1545	1550
Pro Ala Thr Arg Gln Lys Gly Arg Arg Asp Gly Glu Asp Leu Asp Ser		
1555	1560	1565
Gln Gly Asp Gly Ser Ser Gln Pro Asp Thr Ile Ser Ile Ala Ser Arg		
1570	1575	1580
Thr Ser Gln Asn Thr Leu Asp Ser Asp Lys Leu Ser Gly Gly Cys Glu		
1585	1590	1595
Leu Thr Val Val Ile His Asp Phe Thr Ala Cys Asn Ser Asn Glu Leu		1600
1605	1610	1615

032796-132.ST25

Thr Ile Arg Arg Gly Gln Thr Val Glu Val Leu Glu Arg Pro His Asp
 1620 1625 1630
 Lys Pro Asp Trp Cys Leu Val Arg Thr Thr Asp Arg Ser Pro Ala Ala
 1635 1640 1645
 Glu Gly Leu Val Pro Cys Gly Ser Leu Cys Ile Ala His Ser Arg Ser
 1650 1655 1660
 Ser Met Glu Met Glu Gly Ile Phe Asn His Lys Asp Ser Leu Ser Val
 1665 1670 1675 1680
 Ser Ser Asn Asp Ala Ser Pro Pro Ala Ser Val Ala Ser Leu Gln Pro
 1685 1690 1695
 His Met Ile Gly Ala Gln Ser Ser Pro Gly Pro Lys Arg Pro Gly Asn
 1700 1705 1710
 Thr Leu Arg Lys Trp Leu Thr Ser Pro Val Arg Arg Leu Ser Ser Gly
 1715 1720 1725
 Lys Ala Asp Gly His Val Lys Lys Leu Ala His Lys His Lys Lys Ser
 1730 1735 1740
 Arg Glu Val Arg Lys Ser Ala Asp Ala Gly Ser Gln Lys Asp Ser Asp
 1745 1750 1755 1760
 Asp Ser Ala Ala Thr Pro Gln Asp Glu Thr Val Glu Glu Arg Gly Arg
 1765 1770 1775
 Asn Glu Gly Leu Ser Ser Gly Thr Leu Ser Lys Ser Ser Ser Ser Gly
 1780 1785 1790
 Met Gln Ser Cys Gly Glu Glu Glu Gly Glu Glu Gly Ala Asp Ala Val
 1795 1800 1805
 Pro Leu Pro Pro Pro Met Ala Ile Gln Gln His Ser Leu Leu Gln Pro
 1810 1815 1820
 Asp Ser Gln Asp Asp Lys Ala Ser Ser Arg Leu Leu Val Arg Pro Thr
 1825 1830 1835 1840
 Ser Ser Glu Thr Pro Ser Ala Ala Glu Leu Val Ser Ala Ile Glu Glu
 1845 1850 1855
 Leu Val Lys Ser Lys Met Ala Leu Glu Asp Arg Pro Ser Ser Leu Leu
 1860 1865 1870
 Val Asp Gln Gly Asp Ser Ser Ser Pro Ser Phe Asn Pro Ser Asp Asn
 1875 1880 1885
 Ser Leu Leu Ser Ser Ser Ser Pro Ile Asp Glu Met Glu Glu Arg Lys
 1890 1895 1900
 Ser Ser Ser Leu Lys Arg Arg His Tyr Val Leu Gln Glu Leu Val Glu
 1905 1910 1915 1920
 Thr Glu Arg Asp Tyr Val Arg Asp Leu Gly Tyr Val Val Glu Gly Tyr
 1925 1930 1935
 Met Ala Leu Met Lys Glu Asp Gly Val Pro Asp Asp Met Lys Gly Lys
 1940 1945 1950
 Asp Lys Ile Val Phe Gly Asn Ile His Gln Ile Tyr Asp Trp His Arg
 1955 1960 1965
 Asp Phe Phe Leu Gly Glu Leu Glu Lys Cys Leu Glu Asp Pro Glu Lys
 1970 1975 1980
 Leu Gly Ser Leu Phe Val Lys His Glu Arg Arg Leu His Met Tyr Ile
 1985 1990 1995 2000
 Ala Tyr Cys Gln Asn Lys Pro Lys Ser Glu His Ile Val Ser Glu Tyr
 2005 2010 2015
 Ile Asp Thr Phe Phe Glu Asp Leu Lys Gln Arg Leu Gly His Arg Leu
 2020 2025 2030
 Gln Leu Thr Asp Leu Leu Ile Lys Pro Val Gln Arg Ile Met Lys Tyr
 2035 2040 2045
 Gln Leu Leu Leu Lys Asp Phe Leu Lys Tyr Ser Lys Lys Ala Ser Leu
 2050 2055 2060
 Asp Thr Ser Glu Leu Glu Arg Ala Val Glu Val Met Cys Ile Val Pro

032796-132.ST25

2065	2070	2075	2080
Arg Arg Cys Asn Asp Met Met Asn Val Gly Arg Leu Gln Gly Phe Asp			
2085	2090	2095	
Gly Lys Ile Val Ala Gln Gly Lys Leu Leu Leu Gln Asp Thr Phe Leu			
2100	2105	2110	
Val Thr Asp Gln Asp Ala Gly Leu Leu Pro Arg Cys Arg Glu Arg Arg			
2115	2120	2125	
Ile Phe Leu Phe Glu Gln Ile Val Ile Phe Ser Glu Pro Leu Asp Lys			
2130	2135	2140	
Lys Lys Gly Phe Ser Met Pro Gly Phe Leu Phe Lys Asn Ser Ile Lys			
2145	2150	2155	2160
Val Ser Cys Leu Cys Leu Glu Glu Asn Val Glu Asn Asp Pro Cys Lys			
2165	2170	2175	
Phe Ala Leu Thr Ser Arg Thr Gly Asp Val Val Glu Thr Phe Ile Leu			
2180	2185	2190	
His Ser Ser Ser Pro Ser Val Arg Gln Thr Trp Ile His Glu Ile Asn			
2195	2200	2205	
Gln Ile Leu Glu Asn Gln Arg Asn Phe Leu Asn Ala Leu Thr Ser Pro			
2210	2215	2220	
Ile Glu Tyr Gln Arg Asn His Ser Gly Gly Gly Gly Gly Gly Ser			
2225	2230	2235	2240
Gly Ala Ala Ala Gly Val Gly Ala Ala Ala Ala Gly Pro Pro Val			
2245	2250	2255	
Ala Ala Ala Ala Thr Val Ala Ala Pro Ala Ala Ala Ala Pro Pro			
2260	2265	2270	
Ala Arg Ala Gly Ala Gly Pro Pro Gly Ser Pro Ser Leu Ser Asp Thr			
2275	2280	2285	
Thr Pro Pro Cys Trp Ser Pro Leu Gln Pro Arg Ala Arg Gln Arg Gln			
2290	2295	2300	
Thr Arg Cys Gln Ser Glu Ser Ser Ser Ser Ser Asn Ile Ser Thr Met			
2305	2310	2315	2320
Leu Val Thr His Asp Tyr Thr Ala Val Lys Glu Asp Glu Ile Asn Val			
2325	2330	2335	
Tyr Gln Gly Glu Val Val Gln Ile Leu Ala Ser Asn Gln Gln Asn Met			
2340	2345	2350	
Phe Leu Val Phe Arg Ala Ala Thr Asp Gln Cys Pro Ala Ala Glu Gly			
2355	2360	2365	
Trp Ile Pro Gly Phe Val Leu Gly His Thr Ser Ala Val Ile Val Glu			
2370	2375	2380	
Asn Pro Asp Gly Thr Leu Lys Lys Ser Thr Ser Trp His Thr Ala Leu			
2385	2390	2395	2400
Arg Leu Arg Lys Lys Ser Glu Lys Lys Asp Lys Asp Gly Lys Arg Glu			
2405	2410	2415	
Gly Lys Leu Glu Asn Gly Tyr Arg Lys Ser Arg Glu Gly Leu Ser Asn			
2420	2425	2430	
Lys Val Ser Val Lys Leu Leu Asn Pro Asn Tyr Ile Tyr Asp Val Pro			
2435	2440	2445	
Pro Glu Phe Val Ile Pro Leu Ser Glu Val Thr Cys Glu Thr Gly Glu			
2450	2455	2460	
Thr Val Val Leu Arg Cys Arg Val Cys Gly Arg Pro Lys Ala Ser Ile			
2465	2470	2475	2480
Thr Trp Lys Gly Pro Glu His Asn Thr Leu Asn Asn Asp Gly His Tyr			
2485	2490	2495	
Ser Ile Ser Tyr Ser Asp Leu Gly Glu Ala Thr Leu Lys Ile Val Gly			
2500	2505	2510	
Val Thr Thr Glu Asp Asp Gly Ile Tyr Thr Cys Ile Ala Val Asn Asp			
2515	2520	2525	

032796-132.ST25

Met Gly Ser Ala Ser Ser Ser Ala Ser Leu Arg Val Leu Gly Pro Gly
 2530 2535 2540
 Met Asp Gly Ile Met Val Thr Trp Lys Asp Asn Phe Asp Ser Phe Tyr
 2545 2550 2555 2560
 Ser Glu Val Ala Glu Leu Gly Arg Gly Arg Phe Ser Val Val Lys Lys
 2565 2570 2575
 Cys Asp Gln Lys Gly Thr Lys Arg Ala Val Ala Thr Lys Phe Val Asn
 2580 2585 2590
 Lys Lys Leu Met Lys Arg Asp Gln Val Thr His Glu Leu Gly Ile Leu
 2595 2600 2605
 Gln Ser Leu Gln His Pro Leu Leu Val Gly Leu Leu Asp Thr Phe Glu
 2610 2615 2620
 Thr Pro Thr Ser Tyr Ile Leu Val Leu Glu Met Ala Asp Gln Gly Arg
 2625 2630 2635 2640
 Leu Leu Asp Cys Val Val Arg Trp Gly Ser Leu Thr Glu Gly Lys Ile
 2645 2650 2655
 Arg Ala His Leu Gly Glu Val Leu Glu Ala Val Arg Tyr Leu His Asn
 2660 2665 2670
 Cys Arg Ile Ala His Leu Asp Leu Lys Pro Glu Asn Ile Leu Val Asp
 2675 2680 2685
 Glu Ser Leu Ala Lys Pro Thr Ile Lys Leu Ala Asp Phe Gly Asp Ala
 2690 2695 2700
 Val Gln Leu Asn Thr Thr Tyr Tyr Ile His Gln Leu Leu Gly Asn Pro
 2705 2710 2715 2720
 Glu Phe Ala Ala Pro Glu Ile Ile Leu Gly Asn Pro Val Ser Leu Thr
 2725 2730 2735
 Ser Asp Thr Trp Ser Val Gly Val Leu Thr Tyr Val Leu Leu Ser Gly
 2740 2745 2750
 Val Ser Pro Phe Leu Asp Asp Ser Val Glu Glu Thr Cys Leu Asn Ile
 2755 2760 2765
 Cys Arg Leu Asp Phe Ser Phe Pro Asp Asp Tyr Phe Lys Gly Val Ser
 2770 2775 2780
 Gln Lys Ala Lys Glu Phe Val Cys Phe Leu Leu Gln Glu Asp Pro Ala
 2785 2790 2795 2800
 Lys Arg Pro Ser Ala Ala Leu Ala Leu Gln Glu Gln Trp Leu Gln Ala
 2805 2810 2815
 Gly Asn Gly Arg Ser Thr Gly Val Leu Asp Thr Ser Arg Leu Thr Ser
 2820 2825 2830
 Phe Ile Glu Arg Arg Lys His Gln Asn Asp Val Arg Pro Ile Arg Ser
 2835 2840 2845
 Ile Lys Asn Phe Leu Gln Ser Arg Leu Leu Pro Arg Val
 2850 2855 2860

<210> 109

<211> 271

<212> PRT

<213> Homo sapiens

<400> 109

Met Val Leu Ile Lys Glu Phe Arg Val Val Leu Pro Cys Ser Val Gln
 1 5 10 15
 Glu Tyr Gln Val Gly Gln Leu Tyr Ser Val Ala Glu Ala Ser Lys Asn
 20 25 30
 Glu Thr Gly Gly Gly Glu Gly Ile Glu Val Leu Lys Asn Glu Pro Tyr
 35 40 45
 Glu Lys Asp Gly Glu Lys Gly Gln Tyr Thr His Lys Ile Tyr His Leu
 50 55 60

032796-132.ST25

Lys Ser Lys Val Pro Ala Phe Val Arg Met Ile Ala Pro Glu Gly Ser
 65 70 75 80
 Leu Val Phe His Glu Lys Ala Trp Asn Ala Tyr Pro Tyr Cys Arg Thr
 85 90 95
 Ile Val Thr Asn Glu Tyr Met Lys Asp Asp Phe Phe Ile Lys Ile Glu
 100 105 110
 Thr Trp His Lys Pro Asp Leu Gly Thr Leu Glu Asn Val His Gly Leu
 115 120 125
 Asp Pro Asn Thr Trp Lys Thr Val Glu Ile Val His Ile Asp Ile Ala
 130 135 140
 Asp Arg Ser Gln Val Glu Pro Ala Asp Tyr Lys Ala Asp Glu Asp Pro
 145 150 155 160
 Ala Leu Phe Gln Ser Val Lys Thr Lys Arg Gly Pro Leu Gly Pro Asn
 165 170 175
 Trp Lys Lys Glu Leu Ala Asn Ser Pro Asp Cys Pro Gln Met Cys Ala
 180 185 190
 Tyr Lys Leu Val Thr Ile Lys Phe Lys Trp Trp Gly Leu Gln Ser Lys
 195 200 205
 Val Glu Asn Phe Ile Gln Lys Gln Glu Lys Arg Ile Phe Thr Asn Phe
 210 215 220
 His Arg Gln Leu Phe Cys Trp Ile Asp Lys Trp Ile Asp Leu Thr Met
 225 230 235 240
 Glu Asp Ile Arg Arg Met Glu Asp Glu Thr Gln Lys Glu Leu Glu Thr
 245 250 255
 Met Arg Lys Arg Gly Ser Val Arg Gly Thr Ser Ala Ala Asp Val
 260 265 270

<210> 110

<211> 233

<212> PRT

<213> Homo sapiens

<400> 110

Asn Ser Val Leu Asn Ser Asn Ala Ile Lys Asn Leu Pro Pro Pro Leu
 1 5 10 15
 Gly Gly Ala Ala Gly His Pro Gly Ser Ala Val Ser Ala Ala Pro Gly
 20 25 30
 Ile Leu Tyr Pro Gly Gly Asn Lys Tyr Gln Thr Ile Asp Asn Tyr Gln
 35 40 45
 Pro Tyr Pro Cys Ala Glu Asp Glu Glu Cys Gly Thr Asp Glu Tyr Cys
 50 55 60
 Ala Ser Pro Thr Arg Gly Gly Asp Ala Gly Val Gln Ile Cys Leu Ala
 65 70 75 80
 Cys Arg Lys Arg Arg Lys Arg Cys Met Arg His Ala Met Cys Cys Pro
 85 90 95
 Gly Asn Tyr Cys Lys Asn Gly Ile Cys Val Ser Ser Asp Gln Asn His
 100 105 110
 Phe Arg Gly Glu Ile Glu Glu Thr Ile Thr Glu Ser Phe Gly Asn Asp
 115 120 125
 His Ser Thr Leu Asp Gly Tyr Ser Arg Arg Thr Thr Leu Ser Ser Lys
 130 135 140
 Met Tyr His Thr Lys Gly Gln Glu Gly Ser Val Cys Leu Arg Ser Ser
 145 150 155 160
 Asp Cys Ala Ser Gly Leu Cys Cys Ala Arg His Phe Trp Ser Lys Ile
 165 170 175
 Cys Lys Pro Val Leu Lys Glu Gly Gln Val Cys Thr Lys His Arg Arg
 180 185 190

032796-132.ST25

Lys Gly Ser His Gly Leu Glu Ile Phe Gln Arg Cys Tyr Cys Gly Glu
 195 200 205
 Gly Leu Ser Cys Arg Ile Gln Lys Asp His His Gln Ala Ser Asn Ser
 210 215 220
 Ser Arg Leu His Thr Cys Gln Arg His
 225 230

<210> 111
 <211> 212
 <212> PRT
 <213> Homo sapiens

<400> 111
 Asn Ser Val Leu Asn Ser Asn Ala Ile Lys Asn Leu Pro Pro Pro Leu
 1 5 10 15
 Gly Gly Ala Ala Gly His Pro Gly Ser Ala Val Ser Ala Ala Pro Gly
 20 25 30
 Ile Leu Tyr Pro Gly Gly Asn Lys Tyr Gln Thr Ile Asp Asn Tyr Gln
 35 40 45
 Pro Tyr Pro Cys Ala Glu Asp Glu Glu Cys Gly Thr Asp Glu Tyr Cys
 50 55 60
 Ala Ser Pro Thr Arg Gly Asp Ala Gly Val Gln Ile Cys Leu Ala
 65 70 75 80
 Cys Arg Lys Arg Arg Lys Arg Cys Met Arg His Ala Met Cys Cys Pro
 85 90 95
 Gly Asn Tyr Cys Lys Asn Gly Ile Cys Val Ser Ser Asp Gln Asn His
 100 105 110
 Phe Arg Gly Glu Ile Glu Glu Thr Ile Thr Glu Ser Phe Gly Asn Asp
 115 120 125
 His Ser Thr Leu Asp Gly Tyr Ser Arg Arg Thr Thr Leu Ser Ser Lys
 130 135 140
 Met Tyr His Thr Lys Gly Gln Glu Gly Ser Val Cys Leu Arg Ser Ser
 145 150 155 160
 Asp Cys Ala Ser Gly Leu Cys Cys Ala Arg His Phe Trp Ser Lys Ile
 165 170 175
 Cys Lys Pro Val Leu Lys Glu Gly Gln Val Cys Thr Lys His Arg Arg
 180 185 190
 Lys Gly Ser His Gly Leu Glu Ile Phe Gln Arg Cys Tyr Cys Gly Glu
 195 200 205
 Gly Leu Ser Cys
 210

<210> 112
 <211> 149
 <212> PRT
 <213> Homo sapiens

<400> 112
 Asn Ser Val Leu Asn Ser Asn Ala Ile Lys Asn Leu Pro Pro Pro Leu
 1 5 10 15
 Gly Gly Ala Ala Gly His Pro Gly Ser Ala Val Ser Ala Ala Pro Gly
 20 25 30
 Ile Leu Tyr Pro Gly Gly Asn Lys Tyr Gln Thr Ile Asp Asn Tyr Gln
 35 40 45
 Pro Tyr Pro Cys Ala Glu Asp Glu Glu Cys Gly Thr Asp Glu Tyr Cys

[illegible]

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<210> 113
<211> 170
<212> PRT
<213> Homo sapiens
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<400> 113
Cys Ala Ser Pro Thr Arg Gly Gly Asp Ala Gly Val Gln Ile Cys Leu
 1      5      10      15
Ala Cys Arg Lys Arg Arg Lys Arg Cys Met Arg His Ala Met Cys Cys
 20      25      30
Pro Gly Asn Tyr Cys Lys Asn Gly Ile Cys Val Ser Ser Asp Gln Asn
 35      40      45
His Phe Arg Gly Glu Ile Glu Glu Thr Ile Thr Glu Ser Phe Gly Asn
 50      55      60
Asp His Ser Thr Leu Asp Gly Tyr Ser Arg Arg Thr Thr Leu Ser Ser
 65      70      75      80
Lys Met Tyr His Thr Lys Gly Gln Glu Gly Ser Val Cys Leu Arg Ser
 85      90      95
Ser Asp Cys Ala Ser Gly Leu Cys Cys Ala Arg His Phe Trp Ser Lys
 100     105     110
Ile Cys Lys Pro Val Leu Lys Glu Gly Gln Val Cys Thr Lys His Arg
 115     120     125
Arg Lys Gly Ser His Gly Leu Glu Ile Phe Gln Arg Cys Tyr Cys Gly
 130     135     140
Glu Gly Leu Ser Cys Arg Ile Gln Lys Asp His His Gln Ala Ser Asn
 145     150     155     160
Ser Ser Arg Leu His Thr Cys Gln Arg His
 165     170

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<210> 114
<211> 128
<212> PRT
<213> Homo sapiens
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<400> 114
Val Ser Ser Asp Gln Asn His Phe Arg Gly Glu Ile Glu Glu Thr Ile
 1          5          10          15
Thr Glu Ser Phe Gly Asn Asp His Ser Thr Leu Asp Gly Tyr Ser Arg
 20          25          30
Arg Thr Thr Leu Ser Ser Lys Met Tyr His Thr Lys Gly Gln Glu Gly
 35          40          45

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032796-132.ST25

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Ser Val Cys Leu Arg Ser Ser Asp Cys Ala Ser Gly Leu Cys Cys Ala
 50          55          60
Arg His Phe Trp Ser Lys Ile Cys Lys Pro Val Leu Lys Glu Gly Gln
65          70          75          80
Val Cys Thr Lys His Arg Arg Lys Gly Ser His Gly Leu Glu Ile Phe
          85          90          95
Gln Arg Cys Tyr Cys Gly Glu Gly Leu Ser Cys Arg Ile Gln Lys Asp
          100          105          110
His His Gln Ala Ser Asn Ser Ser Arg Leu His Thr Cys Gln Arg His
          115          120          125

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<210> 115
 <211> 84
 <212> PRT
 <213> Homo sapiens

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<400> 115
Gly Gln Glu Gly Ser Val Cys Leu Arg Ser Ser Asp Cys Ala Ser Gly
 1          5          10          15
Leu Cys Cys Ala Arg His Phe Trp Ser Lys Ile Cys Lys Pro Val Leu
          20          25          30
Lys Glu Gly Gln Val Cys Thr Lys His Arg Arg Lys Gly Ser His Gly
          35          40          45
Leu Glu Ile Phe Gln Arg Cys Tyr Cys Gly Glu Gly Leu Ser Cys Arg
          50          55          60
Ile Gln Lys Asp His His Gln Ala Ser Asn Ser Ser Arg Leu His Thr
65          70          75          80
Cys Gln Arg His

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<210> 116
 <211> 149
 <212> PRT
 <213> Homo sapiens
 <400> 116

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Cys Ala Ser Pro Thr Arg Gly Gly Asp Ala Gly Val Gln Ile Cys Leu
 1          5          10          15
Ala Cys Arg Lys Arg Arg Lys Arg Cys Met Arg His Ala Met Cys Cys
          20          25          30
Pro Gly Asn Tyr Cys Lys Asn Gly Ile Cys Val Ser Ser Asp Gln Asn
          35          40          45
His Phe Arg Gly Glu Ile Glu Glu Thr Ile Thr Glu Ser Phe Gly Asn
          50          55          60
Asp His Ser Thr Leu Asp Gly Tyr Ser Arg Arg Thr Thr Leu Ser Ser
65          70          75          80
Lys Met Tyr His Thr Lys Gly Gln Glu Gly Ser Val Cys Leu Arg Ser
          85          90          95
Ser Asp Cys Ala Ser Gly Leu Cys Cys Ala Arg His Phe Trp Ser Lys
          100          105          110
Ile Cys Lys Pro Val Leu Lys Glu Gly Gln Val Cys Thr Lys His Arg
          115          120          125
Arg Lys Gly Ser His Gly Leu Glu Ile Phe Gln Arg Cys Tyr Cys Gly
          130          135          140
Glu Gly Leu Ser Cys
145

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032796-132.ST25

<210> 117
 <211> 107
 <212> PRT
 <213> Homo sapiens

<400> 117
 Val Ser Ser Asp Gln Asn His Phe Arg Gly Glu Ile Glu Glu Thr Ile
 1 5 10 15
 Thr Glu Ser Phe Gly Asn Asp His Ser Thr Leu Asp Gly Tyr Ser Arg
 20 25 30
 Arg Thr Thr Leu Ser Ser Lys Met Tyr His Thr Lys Gly Gln Glu Gly
 35 40 45
 Ser Val Cys Leu Arg Ser Ser Asp Cys Ala Ser Gly Leu Cys Cys Ala
 50 55 60
 Arg His Phe Trp Ser Lys Ile Cys Lys Pro Val Leu Lys Glu Gly Gln
 65 70 75 80
 Val Cys Thr Lys His Arg Arg Lys Gly Ser His Gly Leu Glu Ile Phe
 85 90 95
 Gln Arg Cys Tyr Cys Gly Glu Gly Leu Ser Cys
 100 105

<210> 118
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 118
 Gly Asn Lys Tyr Gln Thr Ile Asp Asn Tyr Gln Pro Tyr Pro Cys
 1 5 10 15

<210> 119
 <211> 22
 <212> PRT
 <213> Homo sapiens

<400> 119
 Leu Asp Gly Tyr Ser Arg Arg Thr Thr Leu Ser Ser Lys Met Tyr His
 1 5 10 15
 Thr Lys Gly Gln Glu Gly
 20

<210> 120
 <211> 21
 <212> PRT
 <213> Homo sapiens

<400> 120
 Arg Ile Gln Lys Asp His His Gln Ala Ser Asn Ser Ser Arg Leu His
 1 5 10 15
 Thr Cys Gln Arg His
 20

032796-132.ST25

<210> 121
<211> 15
<212> PRT
<213> Homo sapiens

<400> 121
Arg Gly Glu Ile Glu Glu Thr Ile Thr Glu Ser Phe Gly Asn Asp
1 5 10 15

<210> 122
<211> 19
<212> PRT
<213> Homo sapiens

<400> 122
Glu Ile Phe Gln Arg Cys Tyr Cys Gly Glu Gly Leu Ser Cys Arg Ile
1 5 10 15
Gln Lys Asp

<210> 123
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> LRP5/HBM amino acid sequence

<400> 123
Met Tyr Trp Thr Asp Trp Val Glu Thr Pro Arg Ile Glu
1 5 10

<210> 124
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> LRP5/HBM amino acid sequence

<400> 124
Met Tyr Trp Thr Asp Trp Gly Glu Thr Pro Arg Ile Glu
1 5 10

<210> 125
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> LRP5/HBM amino acid sequence

032796-132.ST25

<400> 125

Lys Arg Thr Gly Gly Lys Arg Lys Glu Ile Leu Ser Ala
 1 5 10

<210> 126

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> LRP5/HBM amino acid sequence

<400> 126

Glu Arg Val Glu Lys Thr Thr Gly Asp Lys Arg Thr Arg Ile Gln Gly
 1 5 10 15
 Arg

<210> 127

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> LRP5/HBM amino acid sequence

<400> 127

Lys Gln Gln Cys Asp Ser Phe Pro Asp Cys Ile Asp Gly Ser Asp Glu
 1 5 10 15

<210> 128

<211> 266

<212> PRT

<213> Homo sapiens

<400> 128

Met Met Ala Leu Gly Ala Ala Gly Ala Thr Arg Val Phe Val Ala Met
 1 5 10 15
 Val Ala Ala Ala Leu Gly Gly His Pro Leu Leu Gly Val Ser Ala Thr
 20 25 30
 Leu Asn Ser Val Leu Asn Ser Asn Ala Ile Lys Asn Leu Pro Pro Pro
 35 40 45
 Leu Gly Gly Ala Ala Gly His Pro Gly Ser Ala Val Ser Ala Ala Pro
 50 55 60
 Gly Ile Leu Tyr Pro Gly Gly Asn Lys Tyr Gln Thr Ile Asp Asn Tyr
 65 70 75 80
 Gln Pro Tyr Pro Cys Ala Glu Asp Glu Glu Cys Gly Thr Asp Glu Tyr
 85 90 95
 Cys Ala Ser Pro Thr Arg Gly Gly Asp Ala Gly Val Gln Ile Cys Leu
 100 105 110
 Ala Cys Arg Lys Arg Arg Lys Arg Cys Met Arg His Ala Met Cys Cys
 115 120 125
 Pro Gly Asn Tyr Cys Lys Asn Gly Ile Cys Val Ser Ser Asp Gln Asn
 130 135 140

032796-132.ST25

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His Phe Arg Gly Glu Ile Glu Glu Thr Ile Thr Glu Ser Phe Gly Asn
145          150          155          160
Asp His Ser Thr Leu Asp Gly Tyr Ser Arg Arg Thr Thr Leu Ser Ser
          165          170          175
Lys Met Tyr His Thr Lys Gly Gln Glu Gly Ser Val Cys Leu Arg Ser
          180          185          190
Ser Asp Cys Ala Ser Gly Leu Cys Cys Ala Arg His Phe Trp Ser Lys
          195          200          205
Ile Cys Lys Pro Val Leu Lys Glu Gly Gln Val Cys Thr Lys His Arg
          210          215          220
Arg Lys Gly Ser His Gly Leu Glu Ile Phe Gln Arg Cys Tyr Cys Gly
225          230          235          240
Glu Gly Leu Ser Cys Arg Ile Gln Lys Asp His His Gln Ala Ser Asn
          245          250          255
Ser Ser Arg Leu His Thr Cys Gln Arg His
          260          265

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<210> 129

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 129

tggtcagcgg cctggaggat gtggccgcag tggacttcc

39

<210> 130

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 130

ggaagtccac tgcggccaca tcctccaggc cgctgacca

39

<210> 131

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 131

aagctgtact ggacggactc agtgaccaac cgcacgcagg

40

<210> 132

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

032796-132.ST25

<400> 132
cctcgatgcg gttggtcact gagtccgtcc agtacagctt 40

<210> 133
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> complementary synthetic oligonucleotide

<400> 133
atgtactgga cagactggaa ggagacgccc cggattgagc g 41

<210> 134
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> complementary synthetic oligonucleotide

<400> 134
cgctcaatcc ggggcgtctc cttccagtct gtccagtaca t 41

<210> 135
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> complementary synthetic oligonucleotide

<400> 135
atgtactgga cagactgggt tgagacgccc cggattgagc g 41

<210> 136
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> complementary synthetic oligonucleotide

<400> 136
cgctcaatcc ggggcgtctc aaaccagtct gtccagtaca t 41

<210> 137
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> complementary synthetic oligonucleotide

<400> 137

032796-132.ST25

atgtactgga cagactggat tgagacgccc cggattgagc g 41

<210> 138

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 138

cgctcaatcc ggggcgtctc aatccagtct gtccagtaca t 41

<210> 139

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 139

atgtactgga cagactggca ggagacgccc cggattgagc g 41

<210> 140

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 140

cgctcaatcc ggggcgtctc ctgccagtct gtccagtaca t 41

<210> 141

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 141

cggacattta ctggcccaat gtactgacca tcgacctgga gg 42

<210> 142

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 142

cctccaggtc gatggtcagt acattgggcc agtaaattgc cg 42

<210> 143

032796-132.ST25

<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> complementary synthetic oligonucleotide

<400> 143
agctctactg ggctgacgtc aagctcagct tcatccaccg 40

<210> 144
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> complementary synthetic oligonucleotide

<400> 144
cggtgatga agctgagctt gacgtcagcc cagtagagct 40

<210> 145
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> complementary synthetic oligonucleotide

<400> 145
gagtgccctc tactcaccgc tggacatcca ggtgctgagc c 41

<210> 146
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> complementary synthetic oligonucleotide

<400> 146
ggctcagcac ctggatgtcc acgggtgagt agagggcact c 41

<210> 147
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> complementary synthetic oligonucleotide

<400> 147
catgtactgg acagactggg tagagaaccc taaaatcgag tgtgc 45

<210> 148
<211> 45
<212> DNA
<213> Artificial Sequence

032796-132.ST25

<220>

<223> complementary synthetic oligonucleotide

<400> 148

gcacactcga ttttaggggtt ctctaccag tctgtccagt acatg

45

<210> 149

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 149

catctactgg accgagtggg tcggcaagcc gaggatcgtg cg

42

<210> 150

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 150

cgcacgatcc tcggcttgcc gaccactcg gtccagtaga tg

42

<210> 151

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 151

gtacttcacc aacatggtgg accgggcagc caagatcgaa cg

42

<210> 152

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 152

cgttcgatct tggctgcccg gtccaccatg ttggtgaagt ac

42

<210> 153

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

032796-132.ST25

<223> complementary synthetic oligonucleotide

<400> 153

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42

<210> 154

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> complementary synthetic oligonucleotide

<400> 154

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42

<210> 155

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 155

ttttttgtcg accaattcca acgctatcaa g

31

<210> 156

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 156

ttttttgtcg acctgcgcta gtcccacccg c

31

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<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 157

ttttttgtcg accgtgtctt ctgatcaaaa tc

32

<210> 158

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

032796-132.ST25

<400> 158
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<210> 159
<211> 37
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<220>
<223> primer

<400> 159
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<211> 35
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<223> primer

<400> 160
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<212> DNA
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<220>
<223> primer

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<212> DNA
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<220>
<223> primer

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<210> 163
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> primer

<400> 163
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032796-132.ST25

<210> 164
<211> 39
<212> DNA
<213> Homo sapiens

<220>
<223> primer

<400> 164
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<210> 165
<211> 42
<212> DNA
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<220>
<223> primer

<400> 165
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<210> 166
<211> 32
<212> DNA
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<220>
<223> primer

<400> 166
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<210> 167
<211> 33
<212> DNA
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<220>
<223> primer

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<210> 168
<211> 690
<212> DNA
<213> Homo sapiens

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gacaactacc agccgtaccc gtgcgcagag gacgaggagt gcggcactga tgagtactgc 180
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cgaaaacgct gcatgcgtca cgctatgtgc tgccccggga attactgcaa aaatggaata 300
tgtgtgtctt ctgatcaaaa tcatttccga ggagaaattg aggaaaaccat cactgaaagc 360
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032796-132.ST25

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caagtgtgta ccaagcatag gagaaaaggc tctcatggac tagaaatatt ccagcgttgt 600
tactgtggag aaggtctgtc ttgccggata cagaaagatc accatcaagc cagtaattct 660
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<210> 169

<211> 1226

<212> DNA

<213> Homo sapiens

<400> 169

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cagccctttg ttttgacctc tctgagccaa ggccaaaacc cagacaggca gccccacgac 180
ctcagcatcg acatctacag ccggacactg ttctggacgt gcgaggccac caataccatc 240
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cgaggcgacg tgtgacgcca tctgcctgcc caaccagttc cgggtgtgca gcggccagtg 1140
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<210> 170

<211> 934

<212> DNA

<213> Homo sapiens

<400> 170

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ggcctcatcc gccctgtggc cctggtggta gacaacacac tgggcaagct gttctgggtg 180
gacgcggacc tgaagcgcat tgagagctgt gacctgtcag gggccaaccg cctgaccctg 240
gaggacgcca acatcgtgca gcctctgggc ctgaccatcc ttggcaagca tctctactgg 300
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agcgacgagg agggctgccc cgtgtgcctc cgccgcccag tcccctgcg cgcggggtca 720
gtgtgtggac ctgcgcctgc gctgcgacgg cgaggcagac tgtcaggacc gctcagacga 780
ggcggactgt gacgccatct ggccctgccc accagttccg gtgtgcgagc ggccagtgtg 840
tcctcatcaa acagcagtgc gactccttcc ccgactgtat cgacggctcc gacgagctca 900
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032796-132.ST25

<210> 171
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<212> PRT
<213> Homo sapiens

<400> 171
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<210> 172
<211> 19
<212> PRT
<213> Homo sapiens

<400> 172
Trp Cys Cys Cys Gly Leu Phe Arg Gly Val Cys Val Trp Ser Cys Gly
1 5 10 15
Ala Asp Asp

<210> 173
<211> 16
<212> PRT
<213> Homo sapiens

<400> 173
Gly Trp Arg Arg Cys Asp Trp Cys Gly Cys Val Ser Trp Cys Trp Val
1 5 10 15

<210> 174
<211> 32
<212> PRT
<213> Homo sapiens

<400> 174
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Ser Cys Cys Ala Val Asp Val Cys Leu Arg Cys Gly Gly Trp Phe Arg
20 25 30

<210> 175
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<400> 175
Ser Cys Cys Ala Val Asp Val Cys Leu Arg Cys Gly Gly Trp Phe Arg
1 5 10 15

<210> 176
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032796-132.ST25

<213> Homo sapiens

<400> 176

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<210> 177

<211> 16

<212> PRT

<213> Homo sapiens

<400> 177

Val	Leu	Ser	Val	Cys	Glu	Val	Cys	Gly	Gly	Val	Phe	Val	Arg	Arg	Cys
1				5				10						15	

<210> 178

<211> 15

<212> PRT

<213> Homo sapiens

<400> 178

Gly	Met	Trp	Tyr	Trp	Ser	Gly	Arg	Asp	Cys	Ala	Leu	Cys	Trp	Leu
1				5				10					15	

<210> 179

<211> 16

<212> PRT

<213> Homo sapiens

<400> 179

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<210> 180

<211> 13

<212> PRT

<213> Homo sapiens

<400> 180

Val	Val	Cys	Trp	Trp	Cys	Gly	Cys	Arg	Gly	Trp	Trp	Arg
1				5				10				

<210> 181

<211> 16

<212> PRT

<213> Homo sapiens

<400> 181

Cys	Val	Cys	Ala	Ser	Phe	Cys	Cys	Cys	Val	Cys	Gly	Leu	Arg	Leu	Leu
1				5					10					15	

032796-132.ST25

<210> 182
<211> 16
<212> PRT
<213> Homo sapiens

<400> 182
Thr Tyr Glu Val Cys Glu Glu Cys Gly Gly Arg Val Arg Met Trp Val
1 5 10 15

<210> 183
<211> 16
<212> PRT
<213> Homo sapiens

<400> 183
Val Val Val Cys Ala Ser Cys Gly Gln Val Trp His Gly Ser Gly Ala
1 5 10 15

<210> 184
<211> 16
<212> PRT
<213> Homo sapiens

<400> 184
Cys Cys Arg Cys Cys His Cys Trp Asp Cys Glu Trp His Met Cys Val
1 5 10 15

<210> 185
<211> 16
<212> PRT
<213> Homo sapiens

<400> 185
Phe Cys Ala Ser Cys Cys Trp Cys Gly Cys Asp Cys Phe Gly Trp Val
1 5 10 15

<210> 186
<211> 16
<212> PRT
<213> Homo sapiens

<400> 186
Cys Asp Tyr Cys Trp Ser Cys Gly Val Trp Cys Pro Ser Ser Trp Leu
1 5 10 15

<210> 187
<211> 16
<212> PRT
<213> Homo sapiens

<400> 187
Val Tyr Leu Cys Val Trp Cys Gly Ala Ala Arg Phe Gly Cys Tyr Gly

032796-132.ST25

1 5 10 15

<210> 188
<211> 16
<212> PRT
<213> Homo sapiens

<400> 188
Phe Cys Val Cys Gly Cys Cys Trp Cys Trp Cys Ala Ala Cys Trp Cys
1 5 10 15

<210> 189
<211> 16
<212> PRT
<213> Homo sapiens

<400> 189
Val Val Leu Cys Ser Arg Cys Gly Arg Leu Trp Arg Trp Ser Cys Gly
1 5 10 15

<210> 190
<211> 16
<212> PRT
<213> Homo sapiens

<400> 190
Glu Val Arg Gln Val Thr Cys Ile Arg Cys Arg Arg Gly Phe Leu Leu
1 5 10 15

<210> 191
<211> 15
<212> PRT
<213> Homo sapiens

<400> 191
Gly Gly Gly Gly Met Trp Glu Ala Trp Ser Cys Tyr Ala Cys Gly
1 5 10 15

<210> 192
<211> 16
<212> PRT
<213> Homo sapiens

<400> 192
Gly Trp Arg Trp Cys Gly Arg Cys Gly Ala Leu Trp Trp Arg Arg Val
1 5 10 15

<210> 193
<211> 485
<212> DNA
<213> Homo sapiens

032796-132.ST25

<400> 193

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gacacggatg tactcaaagc ggacggggcg atcctcgtcg atttctgggc agagtgggtgc 180
ggtccgaatt ccgtggttct gtgttcgcgt tgtgggcgtt tgtggcgggtg gtcgtgtggg 240
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ggcaaaactga ccgttgcaaa actgaacatc gatcaaaacc ctggcactgc gccgaaatat 360
ggcatccgtg gtatcccgac tctgctgctg ttcaaaaacg gtgaagtggc ggcaaccaa 420
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<210> 194

<211> 485

<212> DNA

<213> Homo sapiens

<400> 194

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gacacggatg tactcaaagc ggacggggcg atcctcgtcg atttctgggc agagtgggtgc 180
ggtccgaatt ccgggtggcg gtggtgtggt cgggtgtggg ctttgtgggtg gcgctgtgtt 240
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gtgggtgcac tgtctaaagg tcagttgaaa gagttcctcg acgctaacct ggcgtaagcg 480
gccgc 485
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<210> 195

<211> 485

<212> DNA

<213> Homo sapiens

<400> 195

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gacacggatg tactcaaagc ggacggggcg atcctcgtcg atttctgggc agagtgggtgc 180
ggtccgaatt ccgaggtgctg gcaggttacg tgtattaggt gtcgtcgggg tttctgtgtg 240
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ggcatccgtg gtatcccgac tctgctgctg ttcaaaaacg gtgaagtggc ggcaaccaa 420
gtgggtgcac tgtctaaagg tcagttgaaa gagttcctcg acgctaacct ggcgtaagcg 480
gccgc 485
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<210> 196

<211> 485

<212> DNA

<213> Homo sapiens

<400> 196

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gacacggatg tactcaaagc ggacggggcg atcctcgtcg atttctgggc agagtgggtgc 180
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ggcaaaactga ccgttgcaaa actgaacatc gatcaaaacc ctggcactgc gccgaaatat 360
ggcatccgtg gtatcccgac tctgctgctg ttcaaaaacg gtgaagtggc ggcaaccaa 420
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032796-132.ST25

gtgggtgcac tgtctaaagg tcagttgaaa gagttcctcg acgctaacct ggcgtaagcg 480
gccgc 485

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<211> 485
<212> DNA
<213> Homo sapiens

<400> 197
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gacacggatg tactcaaaagc ggacggggcg atcctcgctg atttctgggc agagtgggtgc 180
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gccgc 485

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<211> 476
<212> DNA
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gaaatattcc agcgttgta ctgtggagaa ggtctgtctt gccggataca gaaagatcac 420
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<210> 199
<211> 539
<212> DNA
<213> Homo sapiens

<400> 199
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atctgtctcg cctgcaggaa gcgccgaaaa cgctgcatgc gtcacgctat gtgctgcccc 180
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agaagaacca ccttgtcttc aaaaatgtat cacaccaaag gacaagaagg ttctgtttgt 360
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tgtaaacctg tcctgaaaga aggtcaagtg tgtaccaagc ataggagaaa aggtctctcat 480
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<210> 200
<211> 416
<212> DNA
<213> Homo sapiens

<400> 200
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032796-132.ST25

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ggtatcccga ctctgctgct gttcaaaaac ggtgaagtgg cggcaaccaa agtgggtgca 360
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<210> 201

<211> 416

<212> DNA

<213> Homo sapiens

<400> 201

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tccatttgtg aggttggtgag gttgtggagt cggtatcctt ggtcttgggt gactagtggg 180
ccgtgcaaaa tgatcgcccc gattctggat gaaatcgctg acgaatatca gggcaaaactg 240
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ggtatcccga ctctgctgct gttcaaaaac ggtgaagtgg cggcaaccaa agtgggtgca 360
ctgtctaaag gtcagttgaa agagttcctc gacgctaacc tggcgtaagc ggccgc 416
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<210> 202

<211> 422

<212> DNA

<213> Homo sapiens

<400> 202

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tccggttgta ctagtgcggg gtgtggtgct tgggctgagg cgggtagggt ttattgtact 180
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gc 422
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<210> 203

<211> 416

<212> DNA

<213> Homo sapiens

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ggtatcccga ctctgctgct gttcaaaaac ggtgaagtgg cggcaaccaa agtgggtgca 360
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<211> 154

<212> PRT

<213> Homo sapiens

<400> 204

032796-132.ST25

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          20          25          30
Asp Asp Ser Phe Asp Thr Asp Val Leu Lys Ala Asp Gly Ala Ile Leu
          35          40          45
Val Asp Phe Trp Ala Glu Trp Cys Gly Pro Asn Ser Val Val Leu Cys
 50          55          60
Ser Arg Cys Gly Arg Leu Trp Arg Trp Ser Cys Gly Thr Ser Gly Pro
 65          70          75          80
Cys Lys Met Ile Ala Pro Ile Leu Asp Glu Ile Ala Asp Glu Tyr Gln
          85          90          95
Gly Lys Leu Thr Val Ala Lys Leu Asn Ile Asp Gln Asn Pro Gly Thr
          100          105          110
Ala Pro Lys Tyr Gly Ile Arg Gly Ile Pro Thr Leu Leu Leu Phe Lys
          115          120          125
Asn Gly Glu Val Ala Ala Thr Lys Val Gly Ala Leu Ser Lys Gly Gln
          130          135          140
Leu Lys Glu Phe Leu Asp Ala Asn Leu Ala
145          150

```

<210> 205

<211> 154

<212> PRT

<213> Homo sapiens

<400> 205

```

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1          5          10          15
Gly Ser Thr Gly Asp Gly Ser Met Ser Asp Lys Ile Ile His Leu Thr
          20          25          30
Asp Asp Ser Phe Asp Thr Asp Val Leu Lys Ala Asp Gly Ala Ile Leu
          35          40          45
Val Asp Phe Trp Ala Glu Trp Cys Gly Pro Asn Ser Gly Trp Arg Trp
 50          55          60
Cys Gly Arg Cys Gly Ala Leu Trp Trp Arg Arg Val Thr Ser Gly Pro
 65          70          75          80
Cys Lys Met Ile Ala Pro Ile Leu Asp Glu Ile Ala Asp Glu Tyr Gln
          85          90          95
Gly Lys Leu Thr Val Ala Lys Leu Asn Ile Asp Gln Asn Pro Gly Thr
          100          105          110
Ala Pro Lys Tyr Gly Ile Arg Gly Ile Pro Thr Leu Leu Leu Phe Lys
          115          120          125
Asn Gly Glu Val Ala Ala Thr Lys Val Gly Ala Leu Ser Lys Gly Gln
          130          135          140
Leu Lys Glu Phe Leu Asp Ala Asn Leu Ala
145          150

```

<210> 206

<211> 154

<212> PRT

<213> Homo sapiens

<400> 206

```

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1          5          10          15

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032796-132.ST25

Gly Ser Thr Gly Asp Gly Ser Met Ser Asp Lys Ile Ile His Leu Thr
 20 25 30
 Asp Asp Ser Phe Asp Thr Asp Val Leu Lys Ala Asp Gly Ala Ile Leu
 35 40 45
 Val Asp Phe Trp Ala Glu Trp Cys Gly Pro Asn Ser Glu Val Arg Gln
 50 55 60
 Val Thr Cys Ile Arg Cys Arg Arg Gly Phe Leu Leu Thr Ser Gly Pro
 65 70 75 80
 Cys Lys Met Ile Ala Pro Ile Leu Asp Glu Ile Ala Asp Glu Tyr Gln
 85 90 95
 Gly Lys Leu Thr Val Ala Lys Leu Asn Ile Asp Gln Asn Pro Gly Thr
 100 105 110
 Ala Pro Lys Tyr Gly Ile Arg Gly Ile Pro Thr Leu Leu Leu Phe Lys
 115 120 125
 Asn Gly Glu Val Ala Ala Thr Lys Val Gly Ala Leu Ser Lys Gly Gln
 130 135 140
 Leu Lys Glu Phe Leu Asp Ala Asn Leu Ala
 145 150

<210> 207
 <211> 154
 <212> PRT
 <213> Homo sapiens

<400> 207
 Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1 5 10 15
 Gly Ser Thr Gly Asp Gly Ser Met Ser Asp Lys Ile Ile His Leu Thr
 20 25 30
 Asp Asp Ser Phe Asp Thr Asp Val Leu Lys Ala Asp Gly Ala Ile Leu
 35 40 45
 Val Asp Phe Trp Ala Glu Trp Cys Gly Pro Asn Ser Gly Gly Gly Gly
 50 55 60
 Met Ile Trp Glu Ala Trp Ser Cys Tyr Ala Cys Gly Thr Ser Gly Pro
 65 70 75 80
 Cys Lys Met Ile Ala Pro Ile Leu Asp Glu Ile Ala Asp Glu Tyr Gln
 85 90 95
 Gly Lys Leu Thr Val Ala Lys Leu Asn Ile Asp Gln Asn Pro Gly Thr
 100 105 110
 Ala Pro Lys Tyr Gly Ile Arg Gly Ile Pro Thr Leu Leu Phe Lys
 115 120 125
 Asn Gly Glu Val Ala Ala Thr Lys Val Gly Ala Leu Ser Lys Gly Gln
 130 135 140
 Leu Lys Glu Phe Leu Asp Ala Asn Leu Ala
 145 150

<210> 208
 <211> 154
 <212> PRT
 <213> Homo sapiens

<400> 208
 Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
 1 5 10 15
 Gly Ser Thr Gly Asp Gly Ser Met Ser Asp Lys Ile Ile His Leu Thr

032796-132.ST25

```

      20      25      30
Asp Asp Ser Phe Asp Thr Asp Val Leu Lys Ala Asp Gly Ala Ile Leu
      35      40      45
Val Asp Phe Trp Ala Glu Trp Cys Gly Pro Asn Ser Leu Trp Ile Gly
      50      55      60
Pro Gly Asp Gln Gly Leu Phe Arg Arg Phe Val Phe Thr Ser Gly Pro
      65      70      75      80
Cys Lys Met Ile Ala Pro Ile Leu Asp Glu Ile Ala Asp Glu Tyr Gln
      85      90      95
Gly Lys Leu Thr Val Ala Lys Leu Asn Ile Asp Gln Asn Pro Gly Thr
      100      105      110
Ala Pro Lys Tyr Gly Ile Arg Gly Ile Pro Thr Leu Leu Leu Phe Lys
      115      120      125
Asn Gly Glu Val Ala Ala Thr Lys Val Gly Ala Leu Ser Lys Gly Gln
      130      135      140
Leu Lys Glu Phe Leu Asp Ala Asn Leu Ala
      145      150

```

<210> 209

<211> 151

<212> PRT

<213> Homo sapiens

<400> 209

```

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1      5      10      15
Gly Ser Thr Gly Asp Gly Ser Val Ser Ser Asp Gln Asn His Phe Arg
      20      25      30
Gly Glu Ile Glu Glu Thr Ile Thr Glu Ser Phe Gly Asn Asp His Ser
      35      40      45
Thr Leu Asp Gly Tyr Ser Arg Arg Thr Thr Leu Ser Ser Lys Met Tyr
      50      55      60
His Thr Lys Gly Gln Glu Gly Ser Val Cys Leu Arg Ser Ser Asp Cys
      65      70      75      80
Ala Ser Gly Leu Cys Cys Ala Arg His Phe Trp Ser Lys Ile Cys Lys
      85      90      95
Pro Val Leu Lys Glu Gly Gln Val Cys Thr Lys His Arg Arg Lys Gly
      100      105      110
Ser His Gly Leu Glu Ile Phe Gln Arg Cys Tyr Cys Gly Glu Gly Leu
      115      120      125
Ser Cys Arg Ile Gln Lys Asp His His Gln Ala Ser Asn Ser Ser Arg
      130      135      140
Leu His Thr Cys Gln Arg His
      145      150

```

<210> 210

<211> 172

<212> PRT

<213> Homo sapiens

<400> 210

```

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1      5      10      15
Gly Ser Thr Gly Asp Gly Ser Cys Ala Ser Pro Thr Arg Gly Gly Asp
      20      25      30
Ala Gly Val Gln Ile Cys Leu Ala Cys Arg Lys Arg Arg Lys Arg Cys

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032796-132.ST25

```

      50              55              60
Leu Asp Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr Val Ala Lys
65              70              75              80
Leu Asn Ile Asp Gln Asn Pro Gly Thr Ala Pro Lys Tyr Gly Ile Arg
      85              90              95
Gly Ile Pro Thr Leu Leu Leu Phe Lys Asn Gly Glu Val Ala Ala Thr
      100              105              110
Lys Val Gly Ala Leu Ser Lys Gly Gln Leu Lys Glu Phe Leu Asp Ala
      115              120              125
Asn Leu Ala
130

```

<210> 213
 <211> 133
 <212> PRT
 <213> Homo sapiens

```

<400> 213
Met Gly Asp Lys Ile Ile His Leu Thr Asp Asp Ser Phe Asp Thr Asp
1              5              10              15
Val Leu Lys Ala Asp Gly Ala Ile Leu Val Asp Phe Trp Ala Glu Trp
      20              25              30
Cys Gly Pro Asn Ser Gly Cys Thr Ser Ala Val Cys Gly Ala Trp Ala
      35              40              45
Glu Ala Gly Arg Phe Tyr Cys Thr Ser Gly Pro Cys Lys Met Ile Ala
      50              55              60
Pro Ile Leu Asp Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr Val
65              70              75              80
Ala Lys Leu Asn Ile Asp Gln Asn Pro Gly Thr Ala Pro Lys Tyr Gly
      85              90              95
Ile Arg Gly Ile Pro Thr Leu Leu Leu Phe Lys Asn Gly Glu Val Ala
      100              105              110
Ala Thr Lys Val Gly Ala Leu Ser Lys Gly Gln Leu Lys Glu Phe Leu
      115              120              125
Asp Ala Asn Leu Ala
130

```

<210> 214
 <211> 131
 <212> PRT
 <213> Homo sapiens

```

<400> 214
Met Gly Asp Lys Ile Ile His Leu Thr Asp Asp Ser Phe Asp Thr Asp
1              5              10              15
Val Leu Lys Ala Asp Gly Ala Ile Leu Val Asp Phe Trp Ala Glu Trp
      20              25              30
Cys Gly Pro Asn Ser Leu Trp Ile Gly Pro Gly Asp Gln Gly Leu Phe
      35              40              45
Arg Arg Phe Val Phe Thr Ser Gly Pro Cys Lys Met Ile Ala Pro Ile
      50              55              60
Leu Asp Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr Val Ala Lys
65              70              75              80
Leu Asn Ile Asp Gln Asn Pro Gly Thr Ala Pro Lys Tyr Gly Ile Arg
      85              90              95

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032796-132.ST25

```

      35          40          45
Met Arg His Ala Met Cys Cys Pro Gly Asn Tyr Cys Lys Asn Gly Ile
  50          55          60
Cys Val Ser Ser Asp Gln Asn His Phe Arg Gly Glu Ile Glu Glu Thr
  65          70          75          80
Ile Thr Glu Ser Phe Gly Asn Asp His Ser Thr Leu Asp Gly Tyr Ser
      85          90          95
Arg Arg Thr Thr Leu Ser Ser Lys Met Tyr His Thr Lys Gly Gln Glu
      100          105          110
Gly Ser Val Cys Leu Arg Ser Ser Asp Cys Ala Ser Gly Leu Cys Cys
      115          120          125
Ala Arg His Phe Trp Ser Lys Ile Cys Lys Pro Val Leu Lys Glu Gly
      130          135          140
Gln Val Cys Thr Lys His Arg Arg Lys Gly Ser His Gly Leu Glu Ile
      145          150          155          160
Phe Gln Arg Cys Tyr Cys Gly Glu Gly Leu Ser Cys
      165          170

```

<210> 211
 <211> 131
 <212> PRT
 <213> Homo sapiens

```

<400> 211
Met Gly Asp Lys Ile Ile His Leu Thr Asp Asp Ser Phe Asp Thr Asp
  1          5          10          15
Val Leu Lys Ala Asp Gly Ala Ile Leu Val Asp Phe Trp Ala Glu Trp
      20          25          30
Cys Gly Pro Asn Ser Tyr Ala Trp Leu Phe Ser Cys Ser Arg Cys Arg
  40          45          50          55          60          65          70          75          80          85          90          95
Trp Trp Leu Pro Trp Thr Ser Gly Pro Cys Lys Met Ile Ala Pro Ile
  50          55          60          65          70          75          80          85          90          95
Leu Asp Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr Val Ala Lys
  65          70          75          80          85          90          95
Leu Asn Ile Asp Gln Asn Pro Gly Thr Ala Pro Lys Tyr Gly Ile Arg
  85          90          95
Gly Ile Pro Thr Leu Leu Leu Phe Lys Asn Gly Glu Val Ala Ala Thr
      100          105          110
Lys Val Gly Ala Leu Ser Lys Gly Gln Leu Lys Glu Phe Leu Asp Ala
      115          120          125
Asn Leu Ala
      130

```

<210> 212
 <211> 131
 <212> PRT
 <213> Homo sapiens

```

<400> 212
Met Gly Asp Lys Ile Ile His Leu Thr Asp Asp Ser Phe Asp Thr Asp
  1          5          10          15
Val Leu Lys Arg His Gly Ala Ile Leu Val Asp Phe Trp Ala Glu Trp
      20          25          30
Cys Gly Pro Asn Ser Ile Cys Glu Val Val Arg Leu Trp Ser Arg Tyr
      35          40          45
Pro Trp Ser Trp Val Thr Ser Gly Pro Cys Lys Met Ile Ala Pro Ile

```

032796-132.ST25

```

      35          40          45
Met Arg His Ala Met Cys Cys Pro Gly Asn Tyr Cys Lys Asn Gly Ile
  50          55          60
Cys Val Ser Ser Asp Gln Asn His Phe Arg Gly Glu Ile Glu Glu Thr
  65          70          75          80
Ile Thr Glu Ser Phe Gly Asn Asp His Ser Thr Leu Asp Gly Tyr Ser
      85          90          95
Arg Arg Thr Thr Leu Ser Ser Lys Met Tyr His Thr Lys Gly Gln Glu
      100          105          110
Gly Ser Val Cys Leu Arg Ser Ser Asp Cys Ala Ser Gly Leu Cys Cys
      115          120          125
Ala Arg His Phe Trp Ser Lys Ile Cys Lys Pro Val Leu Lys Glu Gly
      130          135          140
Gln Val Cys Thr Lys His Arg Arg Lys Gly Ser His Gly Leu Glu Ile
      145          150          155          160
Phe Gln Arg Cys Tyr Cys Gly Glu Gly Leu Ser Cys
      165          170

```

<210> 211
 <211> 131
 <212> PRT
 <213> Homo sapiens

```

<400> 211
Met Gly Asp Lys Ile Ile His Leu Thr Asp Asp Ser Phe Asp Thr Asp
  1          5          10          15
Val Leu Lys Ala Asp Gly Ala Ile Leu Val Asp Phe Trp Ala Glu Trp
      20          25          30
Cys Gly Pro Asn Ser Tyr Ala Trp Leu Phe Ser Cys Ser Arg Cys Arg
  40          45
Trp Trp Leu Pro Trp Thr Ser Gly Pro Cys Lys Met Ile Ala Pro Ile
  50          55          60
Leu Asp Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr Val Ala Lys
  65          70          75          80
Leu Asn Ile Asp Gln Asn Pro Gly Thr Ala Pro Lys Tyr Gly Ile Arg
      85          90          95
Gly Ile Pro Thr Leu Leu Leu Phe Lys Asn Gly Glu Val Ala Ala Thr
      100          105          110
Lys Val Gly Ala Leu Ser Lys Gly Gln Leu Lys Glu Phe Leu Asp Ala
      115          120          125
Asn Leu Ala
      130

```

<210> 212
 <211> 131
 <212> PRT
 <213> Homo sapiens

```

<400> 212
Met Gly Asp Lys Ile Ile His Leu Thr Asp Asp Ser Phe Asp Thr Asp
  1          5          10          15
Val Leu Lys Arg His Gly Ala Ile Leu Val Asp Phe Trp Ala Glu Trp
      20          25          30
Cys Gly Pro Asn Ser Ile Cys Glu Val Val Arg Leu Trp Ser Arg Tyr
      35          40          45
Pro Trp Ser Trp Val Thr Ser Gly Pro Cys Lys Met Ile Ala Pro Ile

```

032796-132.ST25

```

      50              55              60
Leu Asp Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr Val Ala Lys
65              70              75              80
Leu Asn Ile Asp Gln Asn Pro Gly Thr Ala Pro Lys Tyr Gly Ile Arg
      85              90              95
Gly Ile Pro Thr Leu Leu Leu Phe Lys Asn Gly Glu Val Ala Ala Thr
      100             105             110
Lys Val Gly Ala Leu Ser Lys Gly Gln Leu Lys Glu Phe Leu Asp Ala
      115             120             125
Asn Leu Ala
      130

```

<210> 213
 <211> 133
 <212> PRT
 <213> Homo sapiens

```

<400> 213
Met Gly Asp Lys Ile Ile His Leu Thr Asp Asp Ser Phe Asp Thr Asp
1              5              10              15
Val Leu Lys Ala Asp Gly Ala Ile Leu Val Asp Phe Trp Ala Glu Trp
      20              25              30
Cys Gly Pro Asn Ser Gly Cys Thr Ser Ala Val Cys Gly Ala Trp Ala
      35              40              45
Glu Ala Gly Arg Phe Tyr Cys Thr Ser Gly Pro Cys Lys Met Ile Ala
      50              55              60
Pro Ile Leu Asp Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr Val
65              70              75              80
Ala Lys Leu Asn Ile Asp Gln Asn Pro Gly Thr Ala Pro Lys Tyr Gly
      85              90              95
Ile Arg Gly Ile Pro Thr Leu Leu Leu Phe Lys Asn Gly Glu Val Ala
      100             105             110
Ala Thr Lys Val Gly Ala Leu Ser Lys Gly Gln Leu Lys Glu Phe Leu
      115             120             125
Asp Ala Asn Leu Ala
      130

```

<210> 214
 <211> 131
 <212> PRT
 <213> Homo sapiens

```

<400> 214
Met Gly Asp Lys Ile Ile His Leu Thr Asp Asp Ser Phe Asp Thr Asp
1              5              10              15
Val Leu Lys Ala Asp Gly Ala Ile Leu Val Asp Phe Trp Ala Glu Trp
      20              25              30
Cys Gly Pro Asn Ser Leu Trp Ile Gly Pro Gly Asp Gln Gly Leu Phe
      35              40              45
Arg Arg Phe Val Phe Thr Ser Gly Pro Cys Lys Met Ile Ala Pro Ile
      50              55              60
Leu Asp Glu Ile Ala Asp Glu Tyr Gln Gly Lys Leu Thr Val Ala Lys
65              70              75              80
Leu Asn Ile Asp Gln Asn Pro Gly Thr Ala Pro Lys Tyr Gly Ile Arg
      85              90              95

```

032796-132.ST25

Gly Ile Pro Thr Leu Leu Leu Phe Lys Asn Gly Glu Val Ala Ala Thr
100 105 110
Lys Val Gly Ala Leu Ser Lys Gly Gln Leu Lys Glu Phe Leu Asp Ala
115 120 125
Asn Leu Ala
130

